# CHEMICAL COMPOSITION OF *Cupressus lusitanica*, MILLER AND *Eucalyptus saligna*, SMITH ESSENTIAL OILS AND BIOACTIVITY AGAINST LEPIDOPTERAN AND COLEOPTERAN PESTS OF STORED GRAINS

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**A Thesis Submitted to the Graduate School in Fulfillment for the Requirements for the award of the Degree of Doctor of Philosophy in Applied Entomology of Egerton University.**

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## DECLARATION AND RECOMMENDATION

**Declaration**

I declare that this thesis is my original work and has not been presented in Egerton University or any other university for the award of a degree.

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## DEDICATION

To all my family members

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## ABSTRACT

The chemical composition of *C. lusitanica* and *E. saligna* essential oils was determined and leaf essential oils evaluated for contact and fumigant toxicity, repellence and reproduction inhibition effects against *S. cerealella, A. obtectus, S. zeamais* and *T.castaneum***.** Bioassays were carried out at temperature of 28±2$^{o}$C and relative humidity of 65±5% and laid out in CRD with four replicates per treatment. In all bioassays, essential oils were applied at 0.00, 0.05, 0.10, 0.15 and 0.20% v/w except fumigation. In the instant contact toxicity, oil was applied on wheat and bean or maize grains in 100 ml glass jars. In the residual contact toxicity oils were applied as above but for treated grain storage duration of 30-120 days. In the space fumigation, oils were applied at 0, 5, 10, 15 and 20 µlL$^{-1}$ air in a space fumigation chamber whereas in grain fumigation oil was assayed at 0, 30, 50, 70 and 100 µlL$^{-1}$ air and test insects exposed to oils for 3-10 days. In instant repellency, oils were assayed in an alternate untreated -treated bioassay system whereas in residual repellence oils were assayed as above but treated grain was stored for 30-120 days. In reproductive inhibition test insects were allowed to lay eggs in petri-dishes lined with filter papers soaked in test oils. Leaves yielded the highest amount of oil, 0.31% in *E. saligna* and 0.35% in *C. lusitanica*. In *C. lusitanica* essential oil, β-pinene (38.1 %) α-pinene (23.9 %) β-phellandrene (10.8 %) dominated in fruit, bark and leaves, respectively whereas in *E. saligna p*-cymene (26.8 %), sabinene (12.1%) and borneol (5.1%) dominated in leaves, fruit and bark respectively. In instant and residual contact toxicity, *C. lusitanica* and *E. saligna* essential oil caused mortality of 5-93.0 and 19.7- 89.5 %, respectively. In space and grain fumigation *C. lusitanica* and *E. saligna* essential oils caused mortality of 18.5-100 and 2.3-100 %, respectively. In instant repellence, *C. lusitanica* and *E. saligna* essential oil elicited percentage repellence (PR) values of 30-92.5 and -10-9.3 % but in residual repellence bioassay, oils produced PR values of 37.9-51.1 and 34-52.4%, respectively. Percent progeny reduction in *C. lusitanica* and *E. saligna* essential oils was 50- 100 and 58 - 100 %, respectively. The effects of *C. lusitanica* and *E. saligna* on the insect pests of stored products are manifold, hence promising insecticides and repellents to be used against insect pests of stored grains. Therefore, with more bioactivity studies on more insects and policies in place on formulation and application protocols, the oils might find a place in insect pest control.

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## LIST OF ABBREVIATIONS AND ACRONYMNS

CRD: Completely randomized design

PR: Percent repellence

AGRA: Alliance for Green Revolution in Africa

WEP: World Food Programme

LVB: Lake Victoria Basin

MASL: Meters above sea level

$LD_{50:}$ Lethal dose that kills 50 % of organism

HSD: Honestly significant difference

SPSS: Statistcal Package for social sciences

$LC_{50}$: Lethal concentration that kills 50% of organism

v/w: Volume/weight

GC: Gas chromatography

MS: Mass spectrometry

ANOVA: Analysis of variance

MIC: Minimum inhibition concentration

$^{TM}$: Registered trade mark

DAT: Days after treatment

DEET: N, N-diethyl-*m*-toluamide

IR: Inhibition rates

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# CHAPTER ONE
# INTRODUCTION

## 1.1 Background information

Subsistence farming is a predominant rural activity in most developing countries with 50 to 70% of the population directly engaged (Nukenine, 2010). In the East African region, 70 % of stable food crops are produced by small-scale producers who usually cultivate less than 2.0 hectares (ha) of land using limited technology (Kamatenesi-Mugisha *et al*., 2008; Deng *et al.,* 2009). In Kenya, agriculture contributes about 29.3 per cent to the Gross Domestic product (GDP) and provides employment either directly or indirectly to more than 75 per cent of young men and women in rural areas (AGRA, 2013; WFP, 2014). Despite being important and key to economic development, agricultural productivity in the tropics is constrained by, among others, unreliable rainfall, environmental degradation, diseases and insect pests. Insects cause substantial quantitative and qualitative pre- and post–harvest losses, varying in magnitude from 10 to 60% (Obeng-Ofori *et al*., 2011). In Kenya, insect pests cause 10-100% losses of stored cereal and legume grains depending on the species, storage duration and grain form, pest control practices and whether the grains are stored in central stores or on farm structures (Ogendo *et al*., 2012). Damage due to insects affects mainly the quality, quantity, and commercial value of stored products (Nukenine, 2010).

The grain weevils (*Sitophilus* spp.), Angoumois grain moth, *Sitotroga cerealella* Olivier, bostrichid beetles, *Prostephanus truncatus* Horn and *Rhyzopertha dominica* F., bean bruchid, *Acanthoscelides obtectus* Say, cowpea beetles, *Callosobruchus chinensis* F., Mexican bean weevil, *Zabrotes subfasciatus* Boheman and groundnut borer (*Caryedon serratus* Olivier) have been identified as the major primary insect pests of stored cereal and legume grains in the tropics (Kamatenesi-Mugisha *et al*., 2008; Ogendo *et al*., 2012; Regnault-Roger *et al*., 2012). The rust-red flour beetle *Tribolium castaneum* Herbst, the saw-toothed grain beetle, *Oryzaephilus surinamensis* L., *Cryptolestes* spp., *Trogoderma granarium* Everts and *Cadra cautella* are the major secondary insect pests of food grains in sub-Saharan Africa and the tropics at large (Lee *et al*., 2003; Ogendo *et al*., 2012; Regnault-Roger *et al*., 2012).

In a attempt to effectively control insect pests in field and storage, synthetic insecticides have been used in the past. However, their repeated use to control insect pests, though effective, disrupted biological control activities of natural enemies and led to outbreaks of insect pests, development of resistance, undesirable effects on non-target organisms and humans and environment (Talukder, 2006; Isman, 2007; Philips and Throne, 2010). Furthermore, most small scale farmers in Africa cannot afford modern pest management technologies because of poverty and little formal education (Abate *et al*., 2007; Kamatenesi-Mugisha *et al*., 2008). Additionally, the use of the principal fumigant methyl bromide, has been phased out in many countries because of ozone layer depletion. Phosphine remains the only principal fumigant in bulk storage despite reports of its carcinogenicity and insect resistance (Shaaya and Kostyukovsky, 2006; Batish *et al.*, 2008). In these scenarios, traditional control practices are still the major means of pest management by small-holder farmers in Africa (Kamatenesi-Mugisha *et al*., 2008). However, the scattered information available is mostly observational and does not provide quantitative details about the efficacy of various traditional control methods of insecticides of plant origin particularly essential oils (Kamatenesi-Mugisha *et al*., 2008; Ogendo *et al*., 2012). The outcomes of this new study is envisaged to lead to identification of new important compounds of plant origin that can be used by farmers as alternatives to synthetic insecticide, in protection of stored food grains. This will contribute significantly towards improvement of stored grain insect pest management in subsistence agriculture, which will in turn improve food security, create wealth and spur the rural economic sub-sector development.

### 1.2 Statement of the problem

Smallholder farming in many developing countries is characterized by high poverty levels, cyclic famines and food insecurity. Farmers in these environments have been by-passed by agricultural modernization, mainly because new technologies were availed on unfavorable terms and hence unsuitable to their agro-ecological and socioeconomic conditions. Documented information indicates that insect pest problem is real and one of the greatest hindrances to increased food production and quality storage. Stored grains are estimated to account for 10-40% loss globally due to insect damage and about 10-100% in Kenya. It is in these loss sceneraios that producers are forced to sell grain earlier than they would wish.

Although synthetic pesticides are available, their use has remained largely incompatible with subsistence agriculture because of economic, social, health and environmental concerns. Besides, insecticides are toxic to living organisms and pollute the environment. In addition, intensive use of insecticides increases the chances of pests developing resistance. Methyl bromide and phosphine are the two principal fumigants used world-wide for a long time for the protection of stored food grains. Methyl bromide effects on the ozone layer has resulted in it being phased out globally. Phosphine is still in use despite reports of carcinogenicity and insect resistance. In this respect, there is an urgent need to develop simple, affordable and safer insecticides of plant origin with potential to replace the undesirable synthetic insecticides. There is also an urgent need to fully study the modes of action of essential oils and their efficacy and safety against stored product insect pests, in order to incorporate them in pest management practices, especially in smallholder agriculture.

### 1.3. Objectives

#### 1.3.1 General objective:

Contribute to improved food security and enhanced income for smallholder farmers through rationalized application of selected plant essential oils for insect pest management and thereby reducepost-harvest losses.

#### 1.3.2 Specific objectives

To determine the:

1. Intra- and inter-plant chemical composition of *C. lusitanica* and *E. saligna* essential oils.
2. Instant and residual contact toxicity of *C. lusitanica* and *E. saligna* leaf essential oils on adult stages of *A. obtectus, S. cereallela, S. zeamais* and *T. castaneum;*
3. Space and grain fumigant toxicity of *C. lusitanica* and *E. saligna* leaf essential oils on adult stages of *A. obtectus*, *S. cereallela, S. zeamais* and *T. castaneum;*
4. Instant and residual repellent effects of *C. lusitanica* and *E. saligna* leaf essential oils on adult *A. obtectus*, *S. cereallela, S. zeamais* and *T. castaneum;*

5. Reproduction inhibitory effects of *C. lusitanica* and *E. saligna* leaf essential oils on *A. obtectus*, *S. zeamais*, and *T. castaneum.*

### 1.4 Hypotheses ($H_0$):

1. Leaf, bark and fruits of *C. lusitanica* and *E. saligna* the same yield of essential oil and the that the essential oils from three plant parts have the same chemical compositions;
2. Essential oils from leaves of *C. lusitanica* and *E. saligna* have no instant and residual contact toxicity effects on adult stages of *A. obtectus*, *S. cereallela,* and *T. castaneum;*
3. Essential oils from leaves of *C. lusitanica* and *E. saligna* have no instant and residual fumigant toxicity effects on adult stages of *A. obtectus, S. cereallela,* and *T. castaneum;*
4. *C. lusitanica* and *E. saligna* leaf essential oils have no instant and residual repellent effects on adult stages of *A. obtectus, S. cereallela, S. zeamais* and *T. castaneum;*
5. *C. lusitanica* and *E. saligna* leaf essential oils have no reproduction inhibitory effects on *A. obtectus, S. zeamais,* and *T. castaneum.*

### 1.5 Justification

The four test insects were selected on the basis of being major pests of stored cereals (*T. castaneum*, *S. cerealella* and *S. zeamais*) and beans (*A. obtectus*). Additionally, the insects pests also belong to different orders and families: *T. castaneum* (Coleoptera: Tenebrionidae), *A. obtectus* (Coleoptera: Bruchidae), *S. cerealella* (Lepidoptera: Gelechiidae) and *S. zeamais* (Coleoptera: Curculionidae) with relative variation in morphology and physiological processes. Furthermore, the two plants *C. lusitanica* and *E. saligna* were chosen out of several plants reported to be used by small scale farmers around LVB to protect stored produce against insect pests (Deng *et al*., 2009). Leaf essential oils of the two plants were used in bioactivity studies since essential oils are stored mainly in leaves in the two plants (Regnault-Roger *et al*., 2012)

Grain production forms an important component of subsistence farmer's food and income security. Therefore any efforts that lead to substantial increase in quality and quantity of stored food grains through improved insect pest management and grain quality storage contributes towards food security and improved incomes. The protection of stored grain would benefit

households intending to sell grain at peak prices and those who would otherwise have to purchase grain because of heavy losses. Despite having different chemical structures and modes of action, botanical insecticides, essential oils included are target specific, relatively safe and affordable as they are readily available. The real benefits of botanical insecticides can be best realized in developing countries, where farmers may not afford synthetic insecticides and the traditional use of crude plants and plant derivatives for protection of stored products is long established. Furthermore, the best role of botanicals in the wealthier countries is in public health and organic food production. Therefore, utilization of indigenous pest management technologies is envisaged to stimulate investment in new industrial crops and grain storage with promising multiplier effects on economic development.

### 1.6 Scope and limitations of study

The study entailed determination of chemical composition of leaf, fruit and bark essential oils of *C. lusitanica* and *E. saligna*. In addition, leaf essential oils from the two plants were evaluated for contact (instant and residual) and fumigant (space and grain) toxicity, repellence (instant and residual) and reproduction inhibition against *T. castaneum* (Coleoptera: Tenebrionidae), *A. obtectus* (Coleoptera: Bruchidae), *S. cerealella* (Lepidoptera: Gelechiidae) and *S. zeamais* (Coleoptera: Curculionidae). This proved to be an enormous task given the limitation of time and resources.

The leaf essential oils were used only in bioactivity studies because essential oils are stored in leaves of the two plants and leaves produced the highest yields of oils.. However, it emerged later after GC-MS analysis that the percentage concentration of essential oil constituents known to have contact, fumigant, repellent and reproduction inhibition effects in different pests of stored products varied with plant parts. However, due to time and resource limitations, it was not possible to factor in bioassays that included fruit and bark essential oils. These could form the basis for further in-depth studies on bioactivity of fruit and bark essential oils.

Unsexed adult insects were used in all bioassays owing to difficulty in sexing individual insects. Furthermore, limited time and resource could not allow inclusion of ovicidal and larvicidal

bioassays. In proposal stages of studies, *A. obtectus*, *S. cereallela*, and *T. castaneum* were to be used in bioactivity studies. However, *S. cereallela* could not reproduce in sufficient numbers to allow completion of all bioassays in time. This necessitated the replacement of *S. cereallela* with *S. zeamais.*

In the contact toxicity, repellence and reproduction inhibition bioassay, negative controls consisted of acetone treated and untreated grains but Actelic Super® and crude soya oil served as positive controls. However, a synthetic fumigant was not used in fumigation bioassay as positive control due to unavailability of suitable application equipment and storage facility.

# CHAPTER TWO
# LITERATURE REVIEW

## 2.1 Biology and economic importance of major stored product insect pests

Documented information indicates that insect pest problem is real and one of the greatest hindrances to increased food production and quality storage. The grain weevils (*Sitophilus* spp), Angoumois grain moth, *S. cerealella*, Bostrichids (*P. truncatus* and *R. dominica*), bean bruchid (*A. obtectus*) the cowpea bruchids (*C. maculatus* and *C. chiensis*) have been identified as the major primary insect pests of stored cereal and legume grains in the tropics (Kamatenesi-Mugisha *et al*., 2008; Deng *et al*., 2009). The rust- red flour beetle (*T. castaneum*), the saw-toothed grain beetle *O. surinamensis*, *Cryptolestes* sp. and *T. granarium* are the major secondary insect pests of food grains in sub-Saharan Africa (Ogendo *et al*., 2012). On the basis of seasonal occurrence, distribution and losses caused, *S. cerealella, A.obtectus, S. zeamais* and *T. castaneum* continue to attract research attention owing to their immense economic importance in the East African region. Their biology, distribution and economic importance are reviewed herein below.

### 2.1.1 Bean bruchid (*A. obtectus*)

The bean bruchid, *A. obtectus*, belongs to the family Bruchidae together with cow pea beetles (*C. chinensis* and *C. maculatus*): destructors of stored legume grains. Adult bean bruchids are 3.2 to 4.0 mm long with a conical prothorax, posterior femora bearing a strong-notched tooth (Plate 2.1a). The eggs are usually milky white 0.60 mm x 0.25 mm. The first-instar larva has a yellow head and long legs and second-instar larva is apodous, white, with a brownish head (Ogendo *et al*., 2012). The beetle develops mainly on bean, more rarely on soya bean and lentil. Adults hibernate inside the seeds, where each seed may contain several individuals. It starts to move around in the seed storehouses or in the fields once the temperature reaches 11°C, and flies in dry and sunny weather (21°C). The eggs are deposited in clusters of 2 to 20 on the pods or inside

them, either on the inner side or directly on the seeds with an average fecundity of 40 to 60 eggs (Ogendo *et al*., 2012).

After eclosion, the first instar-larvae penetrate the seed coat, form a cell, and proceed to develop inside the seed. They pass through four instars before pupating. Feeding by the last instar produces the first characteristic circular "windows" that becomes visible externally as insect development progresses. The newly formed adults may remain in the cell for several days before pushing out through the "window" and exiting the seed. Mating occurs immediately, and egg laying soon follows. The life cycle is completed in 28 days and adult longevity is about 12 days. Adult bruchids do not feed; only the larvae cause damage. The bruchid is a major pest of beans in temperate to subtropical regions worldwide. The potential damage to stored grains by this pest is great because the insect can infest grains both pre- and post-harvest, and several larvae can develop in one seed (Ogendo *et al*., 2012).

### 2.1.2 Angoumois grain moth (*S. cerealella*)

*S. cerealella* is a grain-boring moth in the family Gelechiidae. Adult moths are brownish grey, slightly less than 12 mm long, with a long fringe of hairs on the wings. The forewing is yellow-brown and the hind wing tapers abruptly to a point. The wingspan is approximately 12 mm (Plate 2.1b). The minimum life cycle is 28 days at 30$^{o}$C and 75% R.H. Forty to one hundred and fifty (40-150) eggs are laid on the surface of the grain and the maximum growth rate per month is fifty times. The larvae bore into the grain, where they feed and develop entirely inside the kernels. Before pupation, they cut their characteristic circular exit window in the seed coat. The adult moth is short lived and non-feeding stage (Ogendo *et al*., 2012). The moth is a primary colonizer of maize, rice paddy and sorghum in temperate to subtropical regions globally. It infests grains both pre- and postharvest exposing seed tissue to infestation by other insects, bacteria and fungi (Ogendo *et al*., 2012).

### 2.1.3 Red-rust flour beetle (*T. castaneum*)

*T. castaneum* belongs to the family Tenebrionidae. The adult beetle is characterized by its red color, body size of length 2.5-4.5 mm, parallel side and partially dorso-ventrally flattened body (Plate 2.1c). The females lay eggs loosely within their food throughout adult life. The number of eggs laid depends upon temperature, with average of 2.5 and 11 eggs each day at 25$^{o}$C and

32.5$^{o}$C, respectively. Under optimum conditions (35$^{o}$C and 75% RH), larvae emerge from eggs approximately 3 days after oviposition on a diet of wheat. The larvae molt 7 or 8 times (within 13 days) to reach pupal stage that lasts 4.5 days (Ogendo *et al*., 2012). Development from egg to adult takes 20-30 days leading to a rapid population growth. Adults can live for up to six months.

The beetle is found throughout all tropical, sub-tropical and warm temperate areas of the world. It is a secondary pest of a range of commodities especially, cereals but also groundnuts, spices, coffee, cocoa, dried fruit and occasionally, pulses. Heavy infestations can produce undesirable odors and flavors in commodities due to production of quinine from the abdominal and thoracic defense glands of adults (Ogendo *et al*., 2012).

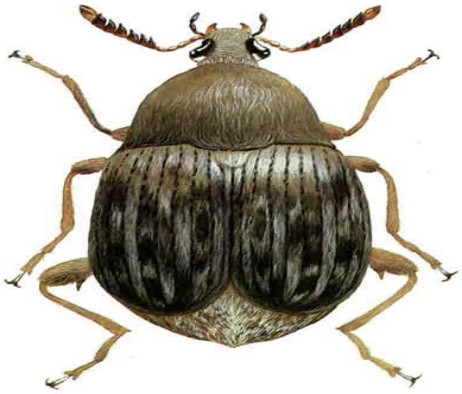
### 2.1.4 Maize weevil (*S. zeamais*)

*S. zeamais* which belongs to the family Curculionidae is 3-4 mm long, brownish- black in color and with a characteristic snout or rostrum (Plate 2.1d). The larvae are 4 mm long, curved in shape and legless. The adult female maize weevil lays her eggs within the actual grain kernels of the maize at a rate of 25 per day, spread over 100 days. The maize weevil bores into the grain with her long snout, inserts her ovipositor into the bored hole and lays a single egg. The eggs hatch in approximately 3 days, depending on the humidity and moisture content of the grain (Ogendo *et al*., 2012).

The larvae, which are approximately 4 mm, white and legless, begin to eat the internal contents of the maize while developing, which takes approximately 18 to 23 days. At this point, they pupate, beginning the transformation into the adult weevil form, much like a butterfly. This process takes approximately 6 days. During these 6 days, the pupae do not eat or move. The weevil then emerges, by cutting a small circular hole in the grain, as an adult and begins the process over again. The entire process takes about 30 to 45 days to complete. The adult maize weevil will also feed on the maize during its lifespan, which is approximately 5 to 8 months long, before dying (Ogendo *et al*., 2012).

*S. zeamais* is a weevil species that is commonly found in maize crops. The larvae damage maize crops by developing within an individual grain, eating it away from the inside out until it

matures, and then reproducing, releasing more crop-damaging larvae. The maize weevil is a danger to both growing standing crops and stored maize (Ogendo *et al.*, 2012).



(a) *Acanthoscelides obtectus*

(b) *Sitotroga cereallela*

(c) *Sitophilus zeamais*

(d) *Tribolium castaneum*

Plate 2.1: Stored product insect pests; (a) *Acanthoscelides obtectus,* (b) *Sitotroga cereallela* (c) *Sitophilus zeamais* and (d) *Tribolium castaneum*

## 2.2 Role of essential oils in stored product insect pest control

Currently the recommended method for protecting stored food grains against insect attack during storage is the application of synthetic organophosphate insecticides. However, considerable problems have emerged from the continued application of these insecticides, including genetic resistance of some insect species, toxic residues on the grains, handling hazards to operator and pest resurgence. Therefore, there is need to replace synthetic insecticides with botanical ones, which are natural in origin and biodegradable, have diverse physiological targets within insects, and consequently, may delay the evolution of insect resistance (Isman, 2007; Kamatenesi-Mugisha *et al*., 2008, Pathipati, 2012).

Since medieval times, plant materials have been used as natural protectants of stored food grains. Several plant parts such as leaves, crushed seeds, powdered fruits and oils among others are examples in this regard (Ogendo *et al*., 2008a; Nukenine 2010; Polatoğlu *et al*., 2011; Regnault-Roger *et al*., 2012). Worldwide research reports have shown that when mixed with stored grains, leaf, bark, seed and powder or oil extracts of plants have reduced oviposition rate and suppressed adult emergence of stored product insects, and have also reduced seed damage rates (Asawalam and Hassanali, 2006; Ogendo *et al*., 2008a). Therefore the search for more refined, selective and biodegradable insecticides is a major target of reserachers currently in stored-product pest management strategy. It is known that tissues of higher plants contain arrays of bioactive-chemicals including essential oils, acids and other compounds that are thought to offer defensive functions (Talukder, 2006; Isman, 2007; Regnault-Roger *et al*., 2012). The composition of essential oils varies with species, season, location, climate, soil type, age of the plant, fertility regime, and the method of oil extraction (Brooker and Kleinig, 2006; Batish *et al.*, 2008; Ogendo, 2008). Essential oils are mainly composed of terpenoids: mono-, sesqui- and di-terpenes and various alcohols, ketones and aldehydes with commonly occurring aromatic compounds arising from the phenylpropanoid pathway (eugenol and safrole). In some species, alkanes, aliphatic alcohols and ketones may be obtained (Batish *et al.*, 2008). Like all the secondary metabolites, essential oils are known to have several important functions, including protection against pathogens (micro-organisms) and predators (insects and herbivores), attraction

of pollinators, inhibition of germination and promotion of plant growth, stimulants or deterrents of feeding and insect oviposition, and insect hormone mimics (Batish *et al.*, 2008; Polatoğlu *et al*., 2011).

On the basis of physiological activities on insects plant essential oils are classified in relation to effects on insects into groups, namely repellents, feeding deterrence/ antifeedants, toxicants, growth and development inhibitors. Repellents from plant origin are considered safe in pest control operations because they minimize pesticide residues; ensure human and wildlife safety, food, environmental stability and wildlife (Talukder, 2004). Plant extracts, powders and essential oil from different bioactive plants have been reported as repellent against different economically important stored product insect pests (Nerio *et al*., 2010; Polatoğlu *et al*., 2011; Nivea *et al*., 2013; Utono *et al*., 2014). The essential oils extracted from aerial parts of *Ocimum americanum*, *Lantana camara* and *Tephrosia vogelli* and monoterpene constituent, eugenol, have also been found to possess concentration, exposure time, species (plant and insect) and plant part-dependent instant and residual repellent potency against adult *T. castaneum*, *R. dominica*, *Sitophilus oryzae* and *C. chinensis* (Ogendo, 2008). The intra- and inter-plant variations in essential oil compositions provide the scientific principles for differential bioactivity responses elicited in the test insects. Repellents are desirable chemicals as they offer protection with minimal impact on the ecosystem by driving away the insect-pests from the treated materials through the stimulation of olfactory or other insect receptors (Nerio *et al*., 2010). Therefore the discovery of a plant-based repellent for the control of stored product insect pests will be a welcome holy grail to plant protection experts.

Antifeedants are of great value in protecting stored commodities from insects. Insects remain on treated food indefinitely and eventually starve to death without feeding (Pungitore *et al*., 2005, Wambua *et al*., 2011). Some naturally occurring antifeedants, which have been characterized, includes glycoside of steroidal alkaloids, aromatic steroids, hydroxylated steroid meliantriol, triterpene hemizectal and others (Isman, 2007). However, not a single crop protection product based unequivocally on feeding or oviposition deterrence has been commercialized. Two main problems face the use of antifeedants in agriculture; interspecific variation in response and

behavioral plasticity in insects-pests which can rapidly habituate to feeding deterrents, rendering them ineffective in a matter of hours (Isman, 2007).

Universal reports on the toxicity of different plant products to stored product insects exists (Rosman *et al*., 2007; Ilboudo *et al*., 2010; Polatoğlu *et al*., 2011; Silva*et al*., 2012; Mishra *et al*., 2014). Studies on plant essential oils and their constituents as fumigants; compounds acting on target insects in the vapour or gaseous phase, against stored-product insects have been reviewed. Fumigant toxicity tests conducted with essential oils of plants (mainly belonging to Apiaceae, Lamiaceae, Lauraceae and Myrtaceae) and their components (cyanohydrins, monoterpenoids, sulphur compounds, thiocyanates and others) have largely focused on beetle pests such as *T. castaneum*, *R. dominica*, *S. oryzae* and *S. zeamais* but little or no attention has been paid towards moths such as *Corcyra cephalonica* and *S. cerealella* (Lee *et al*., 2003; Rajendran and Sriranjini, 2008). Furthermore, essential oils from *L. camara*, *O. americanum* and *T. vogelli* have exhibited contact and fumigant toxicity against *S. zeamais*, *T. castaneum*, *R. dominica*, *S. oryzae* and *C. chinensis*. Insect mortality depended upon rate, formulation, exposure period and plant part used, confirm the existence of moderate to strong concentration, intra-species and inter-plant dependent instant and residual contact toxicity and reproductive inhibitory effects of essential oils (Ogendo, 2008).

Researchers have also reported that essential oils or extracts mixed with stored grains caused reduction in insect oviposition, egg hatchability, post- embryonic development and progeny production (Rajendran and Sriranjini, 2008). In short-term residual bioactivity studies with crude powders and extracts, significant adult insect mortalities and reproduction inhibitory effects against coleopteran pests of stored food commodities have also been reported (Ogendo *et al*., 2008a). Plant extracts showed deleterious effects on the growth and development of insects and reduced larval and pupal adult weight significantly, lengthened the larval and pupal periods and reduced pupal recovery and adult eclosion (Kumar *et al.* 2011). The crude extract also retarded growth, development and caused mortality of larvae, cuticle melanization and high mortality in adults (Koona, 2005). It has also been reported that grains coated with plant extracts completely inhibited the development of insects like *Sitophilus oryzae* (Regnault-Roger *et al*., 2012). Plant extracts also reduce the survival rates of larvae and pupae, and adult emergence. Development

of egg and immature stages inside grain kernels were also inhibited by plant extracts (Kumar *et al.* 2011). Despite of the wide recognition that many plants possess insecticidal, repellent, antifeedant and reproductive inhibition properties, only a handful of pest control products directly obtained from plants are presently in use because of sustainability of the botanical resource, standardization of chemically complex extracts, and regulatory approval. However despite these challenges, several plant essential oils, powders and other extracts have been evaluated against several insect pests of cereals and legumes and found to have contact toxic, repellent, fumigant toxic and antifeedant properties (Rosman *et al*., 2007, Ogendo *et al.*, 2008b). For instance, powders, crude aqueous extracts, essential oils and their constituents from plants in the families, Lamiacae, Verbenaceae, Fabaceae , Leguminosae among others, have shown good bioactivity against a wide range of field and stored-product insect pests (Asawalam *et al.,* 2006; Isman, 2007; Ogendo *et al*., 2011; Wambua *et al*., 2011; Ogendo *et al.*, 2012).

### 2.3 Essential oils of selected plants and insect pest control

Aromatic plants contain volatile compounds, mainly essential oils, known to possess insecticidal properties. The toxic, repellent and reproductive inhibition effects of a large number of essential oils, their chemical constituents of plants such as *Azadirachta indica*, *Mentha* spp. *Cupressus* spp., *Ocimum* spp., *Tithonia* spp. and *Eucalyptus* spp.) have been evaluated against insect pests of crops and have shown promise as control agents (Sim *et al*., 2006; Shaaya and Kostyukovsky, 2006; Kamatenesi-Mugisha *et al*., 2008; Ogendo *et al*., 2008b; Rajendran and Sriranjini, 2008). *C. luistanica* leaves are used in ethno-medicine, aromatherapy, to protect stored grains against insect pests (Kuiate *et al*., 2006; Kamatenesi-Mugisha, et *al*., 2013). The essential oil has also been reported to possess antibacterial and antifungal activity (Hassanzadeh *et al*., 2010). *E. saligna* essential oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/ insect repellent, herbicidal, acaricidal and nematicidal (Batish *et al*., 2008).

#### 2.3.1 Kenya cypress (*C. lusitanica*)

Kenya cypress *Cupressus lusitanica* Miller (Cupressaceae: Pinnales) is an evergreen tree, 25-35 m high, with a dense, conical crown (Plate 2.2a). The distinctly bluish-green foliage is ovate, closely pressed, usually with long, pointed apex. Male flowers small, oblong or cylindrical;

female sub-globose, very small, composed of 6-14 fertile decussate scales with several ovules each. Male cones appear to be fat tips to branchlets; female cones rounded, scales with central pointed projections. Seeds brown, with resin glands, up to 4 mm long, with a narrow wing (Farjon, 2013). *C. lusitanica* is found at altitudes of 1500-4000 Metres Above Sea Level (MASL) and in moist climates, with annual precipitation of 1000 - 1500 mm and a dry season lasting not more than 2-3 months. Its native origin is Central American countries and USA but it has since become an exotic species in other countries including Portugal and East African countries (Katende *et al*., 1995).

*C. lusitanica* is one of the aromatic plants commonly grown and used in local storage structures by communities around the Lake Victoria Basin (LVB) to protect their stored grains against insect infestation (Kamatenesi-Mugisha *et al*., 2008). Their dried leafy branches emit strong persistent aromatic odours for long periods of time, indicating their higher volatile oil content. Essential oils extracted from *Cupressus sempervirens* (species related to *C. lusitanica*) leaves and analyzed by GC-MS has revealed that the oil contained mono-and sesqui-terpenoids with the most important constituents identified as sabinene (14.8%), terpinen-4-ol (11.4%), α-pinene (9.9 %), β-pinene (5.7 %), δ-3-carene (4.2 %), α-terpinene (4.2 %) and limonene (3.9%) (Tapondjou *et al.,* 2005).

Earlier evaluations of *C. lusitanica* essential oils have demonstrated some efficacy against insect pests. For instance, Kanat and Alma (2003) reported that the berry oil was effective in controlling the larvae of pine processionary moth (*Thaumetopoea pityocampa* Schiff). It is, therefore, feasible to use these essential oils from cypress essential oil as environment-friendly insecticides in the control of *T. pityocampa* and possibly other insect pests including stored product pests. Similarly essential oils extracted from *C. sempervirens* leaves have been found to have repellent and toxic effects against *S. zeamais* and *T. confusum* (Tapondjou *et al*., 2005). The oils were toxic to both insects with $LD_{50}$ values of 0.84 and 0.74 $mlcm^{-3}$ for *S. zeamais* and *T. confusum,* respectively. Mortality of *S. zeamais* was almost nil at low concentrations of the essential oil during the first two days but doses of 0.78 $mlcm^{-3}$ of oil was able to induce 100% mortality of insects within 5 days of exposure (Tapondjou *et al*., 2005). The test oils were also highly repellent to the two insect species (PR>70%).

The insecticidal activity of various essential oils has been associated with components such as 1, 8-cineole, citronellal, citronellol, citronellyl acetate, *p*-cymene, eucamalol, limonene, linalool, α-pinene, γ-terpinene, α-terpineol, alloocimene, and aromadendrene (Liu *et al*., 2008; Bachrouch, *et al*., 2010; Sedaghat *et al*., 2011).Therefore, understanding the intra-plant variation in chemical composition (leaves, bark and fruits) and the bio-efficacy of *Cupressus lusitanica* leaf essential oils , against the major insect pests of stored cereal and legume grains requires urgent scientific attention. This will provide useful information on the mechanisms relating to insecticidal, repellent and reproductive inhibition effects of essential oils extracted from the plant against specific stored-product insect pests and use the chemical composition and bio-efficacy of *C. lusitanica* in Lake Victoria basin to make comparisons with what is documented for other regions of the world.

### 2.3.2 Sydney blue gum (*E. saligna*)

Sydney blue gum, *Eucalyptus saligna* Smith (Myrtaceae: Myrtales) is a species of tall, evergreen and magnificent trees native to Australian coastal and lower mountain ranges. *E. saligna* is a woody essential oil-bearing plant, which can grow to a height of 30–65 m (Plate 2.2b). Leaves are discolors, glossy green and thin textured, 10–17 cm long, 2–3 cm wide. Fruits are a small capsule 5-8 mm long, with 3 or 4 valves exerted (Boland *et al*., 2006). Due to its ability to grow at altitudes of 0-1100 MASL and well-drained, deep, loams of alluvial or volcanic origin oils, Sydney blue gum tree has been introduced to many countries including Kenya, UK, and Cameroon among others. Furthermore, it requires annual rainfall of 700-2300 mm and temperature of 10-22 °C. (Boland *et al*., 2006; Slee *et al*., 2006)

*E. saligna* is valued globally for its essential oil, gum, pulp, timber, medicine and aesthetic value. In addition, its essential oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal and nematicidal (Su *et al*., 2006; Batish *et al*., 2008; Liu *et al*., 2008). The eucalyptus essential oil is a complex mixture of monoterpenes and sesquiterpenes, and aromatic phenols, oxides, alcohols, esters, aldehydes and ketones. However, exact composition and proportion, varies with species (Brooker and Kleinig, 2006). The highly toxic and repellent effects of essential oil constituents such as 1, 8-

cineole, terpineol and α-pinene have been demonstrated in various coleopteran pests (Tapondjou *et al*., 2005; Batish *et al.,* 2008). Studies on the bioactivity of essential oils and chemical components of *Eucalyptus* species have also revealed existence of fumigant and repellent properties against permethrin-resistant head lice**.** Assays of individual oil components indicated the vapors of 1, 8-cineole and anisole were the most active, followed by limonene, linalool, menthone, pulegone, myrcene, and benzyl alcohol (Toloza *et al*., 2006; Batish *et al.,* 2008).

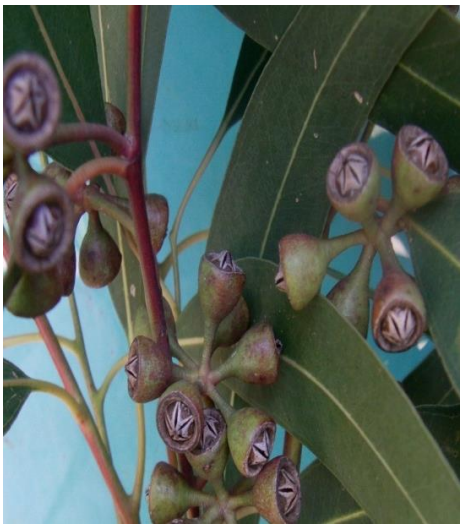
The essential oils extracted from *E. saligna* have been found to have repellent and toxic effects on *S. zeamais* and *T. confusum* (Tapondjou *et al*., 2005). The essential oil was mainly composed constited of α-pinene (39.47%), cymol (31.1%), 1, 8-cineole (9.8 %), terpinene (9.5%) and terpineol (3.7%). Comparison of $LD_{50}$ values for the oils against both insect species showed that Eucalyptus oil was toxic to *Sitophilus zeamais* ($LD_{50}$=0.36 $mlcm^{-3}$) and *T. castaneum* ($LD_{50}$ = 0.48 $mlcm^{-3}$). The lowest dosage of cymol (0.78 $mlcm^{-3}$) induced no mortality of *T. castaneum* within 5 days of exposure while the highest dose of 1.30 $mlcm^{-3}$ induced total mortality after one day (Tapondjou *et al*., 2005). Mortality of *S. zeamais* was almost nil at low concentrations of the essential oil during the first two days but a dose of 1.56 $mlcm^{-3}$ of each oil was able to induce 100% mortality of insects within 5 days of exposure. The test oil was highly repellent to the two insect species (PR>70%) compared to cymol that had a significantly lower repellency effect (PR=60%) (Tapondjou *et al*., 2005).

*E. saligna* essential oil is also used as insect repellent and insecticidal agent (Brooker and Kleinig 2006). The insecticidal activity of eucalyptus oils has been associated with components such as 1, 8-cineole, citronellal, citronellol, citronellyl acetate, *p*-cymene, eucamalol, limonene, linalool, α-pinene, γ-terpinene, α-terpineol, alloocimene, and aromadendrene (Su *et al*., 2006; Batish *et al*., 2008; Liu *et al*., 2008). However, bioactivity and chemical composition of essential oils varies with species, season, location, climate, soil type, and age of the leaves, fertility regime, the method used for drying the plant material, and the method of oil extraction (Brooker and Kleinig, 2006).

Similarly, understanding the intra-plant variation in chemical composition (leaves, bark and fruits) and the bioactivity of leaf essential oils of *E. saligna*, against the major insect pests of

stored cereal and legume grains requires urgent scientific devotion. This will provide useful information on the mechanisms relating to insecticidal, repellent and reproductive inhibition effects of essential oils extracted from the plant against specific stored-product insect pests and use the chemical composition and bio-efficacy of *E. saligna* in the Lake Victoria Basin (LBV) to make comparisons with what is documented for other regions of the world.

(a) *Cupressus lusitanica*



(b) *Eucalyptus saligna*

Plate 2.2: Test insecticidal plants (a) *Cupressus lusitanica* and (b) *Eucalyptus saligna*

# CHAPTER THREE
# GENERAL MATERIALS AND METHODS

## 3.1 Experimental conditions and rearing of test insects

Bioassays were conducted at the Integrated Biotechnology Laboratory at Egerton University, Kenya under controlled conditions of temperature (28±2$^{o}$C), relative humidity (65±5%), and 24h darkness. Clean dry wheat, maize and bean grains, used for experiments, were placed in aluminium foil and kept in the oven at 100$^{o}$C for 24 h to eliminate any latent insect infestation. The experimental and culture room was fitted with a humidifier, automated heating unit and a thermo hygrometer calendar. Adult *S. cerealella, S. zeamais* and *A. obtectus* were reared on whole maize, wheat and bean grains, respectively whereas adult *T. castaneum* were reared on broken wheat grains plus 5% brewer's yeast. In order to secure adults of the same age, all emerging adults were collected daily and put together in rearing jars for 0-5 days for *A. obtectus* and 5-10 days for *S. cerealella, S. zeamais and T. castaneum* prior to use in bioassays. The experimental design was a completely randomized design (CRD) with four replicates per concentration in all bioassays. The small doses of essential oils applied in all bioassays were measured using a micro-pipette.

## 3.2 Statistical data analysis

Insect mortality data were corrected for natural mortality using Abbott's (1925) formula. Data on percentage yield (v/w) of plant part essential oils, percentage insect mortality, repellence and progeny reduction were corrected for heterogeneity of treatment variances using arcsine-transformation (Leatemia and Isman, 2004) before being subjected to one-way ANOVA using JMP 9 software (SAS, 2010). Means were separated by the Tukey-Kramer honestly significant difference (HSD) test at the 5% ($P< 0.05$) significance level (Sokal and Rohlf, 1995). The relationship between the essential oil concentration applied and percentage insect mortality was determined using probit regression analysis of transformed (log base 10) data to estimate lethal concentration that kills 50% ($LC_{50}$) of test insects (SPSS, 2010). Any two $LC_{50}$ values in a column whose 95% confidence limits did not overlap were regarded as significantly different (Finney, 1971). The summaries of statistical analysis output are presented in Appendices 1-11.

# CHAPTER FOUR

# CHEMICAL COMPOSITION OF LEAF, FRUIT AND BARK ESSENTIAL OILS OF *Cupressus lusitanica* Miller AND *Eucalyptus saligna* Smith GROWING IN BUSIA, KENYA


## Abstract

Essential oils from aerial parts of *C. lusitanica* and *E. saligna* plants growing in Busia, Kenya were extracted by hydro-distillation. Leaves yielded the highest amount of oil at 0.35 and 0.31 % in *C. lusitanica* and *E. saligna*, respectively. GC-MS analysis of *C. lusitanica* oils showed that monoterpene hydrocarbons β-phellandrene (10.8%) terpinen-4-ol (9.7%) and δ-3-carene (8.4%), dominated in leaves; β-pinene (38.1%), α-pinene (10.5%), and *p*-cymen-8-ol (5.9%) in fruit and, α-pinene (23.9%) (10.7%) and δ-3-carene (6.9%), β-pinene in bark. In *E. saligna* oil, *p*-cymene (26.8%), α-pinene (14.7%), and α-terpineol (6.0%) in leaves; sabinene (12.1%), terpin-4-ol (9.3%) and δ-3-carene (9.2%) in fruit and borneol (5.1%), α-terpineol (5.1%) and α-2-carene (1.5%), in bark were major constituents. Results of the present study give hope for clear chemical profiling and basis for bioactivity guided development of newer and more selective natural insecticides and other products in related industries.



**Key words**: Chromatography, Hydro-distillation, mass spectra, monoterpenes, sesquiterpenes


## 4.1 Introduction

The two aromatic plants, Kenyan cypress, *Cupressus lusitanica* Mill (Cupressaceae: Pinnales) and Sydney blue gum, *Eucalyptus saligna* Smith (Myrtaceae: Myrtales) are widely cultivated around the world as sources of fuelwood, electric poles, fencing posts, timber, and /or for ornamental purposes, shade and windbreaks (Kuiate *et al*., 2006). However, in recent years, scientists have been evaluating these plants to be used in protection of stored grains from insect infestation, pharmaceuticals and aromatherapy among other uses (Kamatenesi-Mugisha *et al*., 2013; Teke *et al.*, 2013). *C. lusitanica* leaves have been used to cure some skin diseases caused by dermatophytes, to alleviate coughs and cold symptoms and to repel insects from stored grains (Kuiate *et al*., 2006). In addition, the essential oil from *C. lusitanica* is used in aromatherapy and for massage to restore calmness, soothe anger, improve blood circulation, and to treat coughs and bronchitis (Kamatenesi-Mugisha, *et al*., 2013). In other studies, the *C. lusitanica* essential oil has

been reported to possess antibacterial activity against *Bacillus cereus* and antifungal activity against *Aspergillus niger* (Hassanzadeh *et al*., 2010). The essential oil composition of *C. lusitanica* has been studied in; Argentina (Floreani *et al*., 1982), Portugal (Carmo and Frazo, 1989; Adams, 1997), Cameroon (Kuiate *et al*., 2006) and Costa Rica (Hassanzadeh *et al.*, 2010) and reported to contain mainly α-pinene, δ-3-carene, limonene and umbellulone.

Eucalytpus species, including *E. saligna*, on the other hand, not only provide fuel biomass, building materials and reduce atmospheric carbon dioxide levels directly (Liu *et al*., 2008), but also perform a variety of indirect services through their essential oil used as insect pest repellent and as a pesticidal agent (Batish *et al*., 2008). Among the various non-wood products, essential oil found in its foliage is the most important one and has found extensive use in food, perfumery and pharmaceutical industry (Brooker and Kleinig, 2006). In addition, the oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/ insect repellent, herbicidal, acaricidal and nematicidal (Batish *et al*., 2008). The eucalyptus oil is a complex mixture of a variety of monoterpenes and sesquiterpenes, and aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones. However, the exact composition and proportion varies with species (Brooker and Kleinig, 2006; Hermann, 2010).

The variability in composition of the essential oils of *E. saligna* has been studied in Cameroon (Tedonkeng *et al.,* 2004; Tapondjou *et al*., 2005; Dongmo *et al.*, 2008) and Argentina (Alzogaray *et al.*, 2011) and reported to contain mainly α-pinene, 1, 8-cineole (eucalyptol), *p*-cymene (cymol) γ-terpinene and terpinen-4-ol. The pesticidal activity of eucalyptus oils has been attributed to components such as 1, 8-cineole, citronellal, citronellol, citronellyl acetate, *p*-cymene, eucamalol, limonene, linalool, α-pinene, γ-terpinene, α-terpineol, alloocimene, and aromadendrene ( Batish *et al*., 2006; Su *et al*., 2006; Liu *et al*., 2008). However, number and concentration of essential oils varies with species, season, location, climate, soil type, and age of the plants, fertility regime, the method used for drying the plant material, and the method of oil extraction (Brooker and Kleinig, 2006). Therefore, it is of scientific interest to study essential oils from plants which have been used since antiquity, as potential sources of novel antimicrobial compounds and pesticidal agents. The study of the chemical composition of regional aromatic plants with medicinal and pest management importance including *C. lusitanica* and *E. saligna* in

the Eastern African region has received little research attention. In an effort to fill this void, a study was conducted to determine the chemical composition of essential oils obtained from the aerial parts of *C. lusitanica* and *E. saligna* plants growing in Busia, Kenya.

## 4.2 Materials and methods

### 4.2.1 Collection and preparations of plant materials

Samples of fresh leaves, bark and fruits of *C. lusitanica* and *E. saligna* (Plate 2.2) were separately collected from branches of 7 year old trees from Kenya Forestry Services demonstration plots in Busia, (0°27'20.02"N, 34°7'48.00"E, 1216 MASL), Kenya in August, 2012. On the spot identification of *C. lusitanica* and *E. saligna* species was carried out with the help of expertise, pictorial aids and literature materials (Kokwaro and Johns, 1998). Preserved specimens were forwarded to Prof Samuel T. Kariuki, a Plant Taxonomist, Department of Biological Sciences, Egerton University for authentic identification. The fresh leaf samples were air-dried under shade at ambient temperature (18-28°C) for 14 days and further oven dried at 35°C for 48 hours. Dry leaf materials were then ground to fine powder using an electric hammer mill (Wambua *et al*., 2011).

### 4.2.2 Hydro-distillation of essential oils, analysis and identification of essential oil constituents

The powdered material (500 g) of *C. lusitanica* and *E. saligna* leaves, fruit and bark were hydro-distilled using a modified Clevenger-type apparatus (Plate 4.1) for 4 hours and the floating oil which separated from water, was collected. The oil was then dried over anhydrous sodium sulphate and stored in the refrigerator at below 4 °C until use. Each test essential oil (1µl) from the different plants was analyzed by gas chromatography (GC) coupled to mass spectrometry (MS) at the laboratories of the International Centre of Insect Ecology and Physiology (ICIPE), Nairobi on an HP-7890A (Agilent Technologies, Wilmington, USA) GC connected to an HP 5975 C (Agilent, Wilmington, USA) MS. The GC equipment was fitted with a non-polar HP-5MS capillary column (30 m × 0.25 mm internal diameter; 0.25 µm film thickness with 5%-phenyl methyl silicone as the stationary phase (J & W Scientific, Folsom, USA). The carrier gas was Helium (1.2 ml $min^{-1}$); oven temperature programmed at 35°C (for 5 min) to 280°C at 10°C

min−1 and then held isothermal at 280°C for 10.5 min.; injection mode was splitless. Mass spectra were acquired at 70 eV within a mass range of 38–550 Daltons (Da) with a scan time of 0.73 scans $s^{-1}$ whereas the ion source was maintained at a temperature of 230°C. The essential oil constituents were identified by comparing mass spectra with library data (NIST05a and Adams MS HP, USA) and by comparison of the retention times with mass spectra.


Plate 4.1 Modified Clevenger-type apparatus

## 4.3 Results

### 4.3.1 Chemical composition of essential oil of *C. lustanica*

The results of *C. lustanica* and *E. saligna* essential oil extraction indicated that plant parts significantly influenced the percentage yield (v/w) of essential oils (ANOVA: $F_{(1, 2)} = 26.069$; $P < 0.001$). The percentage yields (v/w) of essential oil extraction indicated that *C. lustanica* had leaves were richer (0.35%) in essential oils than the fruits (0.16%) and bark (0.13%).

Table 4.1 shows the retention time (min), identified chemical constituents and percentage concentrations of oils obtained from *C. lusitanica.* In the leaf essential oil, 54 compounds were identified, corresponding to 93.8% of the total essential oil composition. The leaf essential oil contained 79.0, 12.7, 2.1% monoterpenes, sesquiterpenes and diterpenes, respectively. The major monoterpenes were; β-phellandrene (10.8%), terpinen-4-ol (9.7%) and δ-3-carene (8.4%) while sesquiterpenes were mainly *trans*-muurola-4(14), 5-diene (2.9%), α- cedrene (2.6%) and *cis*-cadina-1(6), 4-diene (4.2%). On the other hand, thirty two (32) compounds corresponding to 93.6% were identified in *C. lustanica* fruit essential oil. The fruit essential oil contained relatively high amounts of monoterpenes (89.5%) as compared to sesquiterpenes (3.2%). Fruit essential oil had β-pinene (38.1%) and 1R-α-pinene (10.5%) as major monoterpenes followed by *p*-cymen-8-ol (5.9%) while sesquiterpenes were mainly sibirene (1.2%) and γ-elemene (0.6%).

In the bark essential oil 41 compounds were identified corresponding to 83.0% of the total essential oil composition. Monoterpenes α-pinene (23.9%), β-pinene (10.7%) and δ-3-carene (6.9 %) were detected in bark as major components while sesquiterpenes were mainly (*E*-) caryophyllene (1.4%) and α-humulene (1.9%). For sesquiterpenes, the highest percentages was detected in leaves (10.6%) followed by bark (8.8%) and the smallest amounts were in fruit (3.2 %). Diterpenes were only present in small quantities in fruit and bark essential oils. The principal monoterpenes common in leaf, fruit and bark essential oil were δ-3-carene, α-pinene, γ-terpinene α-terpinene, terpineol and *p*-mentha-1, 5-dien-8-ol (Table 4.1). As for sesquiterpenes, (*E*-) caryophyllene and α-humulene were common in both fruit and bark essential oil. The chemo types of *C. lusitanica* essential oil obtained from Busia were identified as: leaves, β-phellandrene (10.8%); fruit and bark, α-pinene (23.9-38.1%)

Table 4.1: Concentration (% v/w) and retention time (min.) of chemical constituents of *C. lustanica* leaf, fruit and bark essential oils obtained from Busia.

| No | RT (min) | Compound name | % Concentration | | |
|---|---|---|---|---|---|
| | | | Leaves | Fruits | Bark |
| 1 | 7.9 | 2-hexenal | 0.1 | - | - |
| 2 | 9.02 | Heptanol<2-> | 0.2 | - | - |
| 3 | 9.45 | Tricyclo[2.2.1.0(2,6)] heptane, 1,7,7-trimethyl- | - | 0.4 | - |
| 4 | 9.85 | Tricyclene | 0.2 | - | - |
| 5 | 9.99 | α-Phellandrene | 1.6 | 0.4 | - |
| 6 | 10.12 | α-Pinene | 5.8 | 10.5 | 23.9 |
| 7 | 10.43 | α-Fenchene | 1.5 | - | - |
| 8 | 10.55 | Thuja-2,4(10)-diene | - | - | 0.1 |
| 9 | 10.64 | Bicyclo [3.1.1] heptan-3-ol, 6, 6-dimethyl-2-methylene-, [1S-(1.α, 3.α, 5.α)]- | - | 3.5 | 1.3 |
| 10 | 10.72 | β- Pinene | - | 38.1 | 10.7 |
| 11 | 10.95 | β-Myrcene | - | 3.5 | |
| 12 | 11.29 | Myrcene | 3 | - | 3.5 |
| 13 | 11.55 | α-Terpinene | 2.1 | 3.3 | 0.8 |
| 14 | 11.58 | β-Phellandrene | 10.8 | 3.5 | - |
| 15 | 11.66 | δ-3-Carene | 8.4 | 1.5 | 6.9 |
| 16 | 11.76 | *P*-Mentha-1(7),8-diene | 4.2 | - | - |
| 17 | 11.8 | δ-2-Carene | - | 4.7 | 5.4 |
| 18 | 11.95 | *o*-Cymene | 1.9 | - | - |
| 19 | 12.02 | Limonene | - | - | 2.7 |
| 20 | 12.07 | (*E*)-β->Ocimene | 0.1 | - | 0 |
| 21 | 12.1 | 1,8-Cineole | 4.2 | - | - |
| 22 | 12.17 | (*Z*)-β-Ocimene | 0.5 | - | 0.5 |
| 23 | 12.55 | γ-Terpinene | 2.8 | 0.4 | 0.1 |
| 24 | 12.71 | *trans*- (Sabinene hydrateIPP vs OH) | 0.5 | - | - |
| 25 | 13.25 | Linalool | 2.7 | - | - |
| 26 | 13.28 | *cis*-Thujone | 0.5 | - | - |
| 27 | 13.48 | 1,3,8-*p*-Menthatriene | 0.2 | - | - |
| 28 | 13.72 | α–Campholenal | - | 0.7 | - |
| 29 | 14.03 | Camphor | 1.7 | 1 | - |

Table 4.1cont'd

| RT (min) | Compound name | % Concentration | | |
|---|---|---|---|---|
| | | **Leaves** | **Fruits** | **Bark** |
| 14.06 | Carvacrol | - | - | 2.9 |
| 14.11 | Camphene | - | 1.9 | 1.8 |
| 14.15 | *p*-Mentha-1,5-dien-8-ol | 1 | 1.3 | - |
| 14.39 | Borneol | - | 1.5 | - |
| 14.51 | *p*-Cymen-8-ol | 4.4 | 5.9 | - |
| 14.51 | Umbellulone | - | 0.6 | 3.8 |
| 14.53 | Myrtenol | - | 0.5 | - |
| 14.54 | Terpinen-4-ol | 9.7 | 1.4 | - |
| 14.58 | *o*-Cumenol | | | 3.5 |
| 14.76 | *cis*-Piperitol | 0.5 | - | - |
| 15.00 | Citronellol | 1.2 | - | 1 |
| 15.06 | Verbenone | - | 0.8 | 0.7 |
| 15.2 | Carvacrol, methyl ether | - | - | 0.7 |
| 15.41 | Car-3-en-2-one | 0.2 | - | - |
| 15.85 | Bornyl acetate | 0.9 | 0.5 | 0.8 |
| 15.9 | 2-Undecanone | 0.7 | - | - |
| 16.1 | *endo*-Arbozol | 0.9 | - | - |
| 16.33 | Terpinolene | 1.2 | 3.5 | 3.2 |
| 17.58 | Mayurone | 0.5 | - | - |
| 17.74 | Amorpha-4,11-diene | 0.5 | - | - |
| 17.82 | (*E*-)Caryophyllene | - | 0.6 | 1.4 |
| 17.82 | γ-Elemene | - | 0.3 | - |
| 17.85 | 3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde | - | - | 1 |
| 17.98 | α- Cedrene | 0.5 | - | - |
| 18.07 | *cis*-Cadina-1(6),4-diene | 1.6 | - | - |
| 18.38 | Citronellyl butanoate | 0.2 | - | - |
| 18.6 | α-Humulene | | 1.2 | 1.9 |
| 18.61 | *trans*-Muurola-4(14),5-diene | 2.9 | - | - |
| 18.76 | α-Curcumene | 0.3 | - | - |
| 18.88 | β-Alaskene | 0.6 | - | - |
| 19.01 | Epizonarene | 1.4 | - | - |
| 19.17 | Sibirene | - | 0.4 | - |
| 19.41 | *Z*-Nerolidol | 0.3 | - | - |
| 19.80 | Caryophyllene oxide | - | - | 1.1 |

Table 4.1cont'd

| RT (min) | Compound name | % Concentration | | |
|---|---|---|---|---|
| | | **Leaves** | **Fruits** | **Bark** |
| 19.81 | α-Funebrene | 0.8 | - | - |
| 19.97 | α–Caryophyllene | - | - | 1 |
| 20.04 | Cedrol | 0.6 | - | - |
| 20.31 | Italicene | | 0.7 | - |
| 20.33 | α–Acoradiene | - | - | 1.3 |
| 20.75 | *cis*-Cadina-1(6),4-diene | - | - | 0.5 |
| 21.09 | 2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene | - | - | 0.9 |
| 21.27 | 1,7,7-Trimethyl-2-vinylbicyclo [2.2.1]hept-2-ene | - | - | 0.7 |
| 21.34 | *Z*-Nuciferol | 0.5 | | - |
| 23.94 | Kaur-15-ene | - | - | 0.4 |
| 23.98 | Kaur-15-ene, (5.alpha., 9.alpha.,10.beta.)- | 0.9 | - | - |
| 24.26 | Sandaracopimarinal | - | - | 1.1 |
| 24.45 | Phyllocladene | 0.1 | - | - |
| 24.52 | 13-*epi*-Manool oxide | 0.2 | - | - |
| 25.37 | Abietatriene | 0.6 | - | 1.5 |
| 25.81 | Nezukol | 0.2 | - | - |
| | **Total identified (%)** | **93.8** | **93.6** | **83.0** |
| | Monoterpene hydrocarbons | 39.9 | 70.9 | 54.4 |
| | Oxygenated monoterpenes | 37.7 | 18.1 | 16.9 |
| | Sesquiterpenes | 10.6 | 2.1 | 9.0 |
| | Diterpenes | 2.0 | 0 | 3.0 |
| | Others | 3.6 | 2.0 | 0.8 |
| | Essential oil (%) yield (Mean ± S.E) | [a]0.36±0.07 | 0.16±0.02 | 0.13±0.0) |

Column used: HP-5MS

- = Absent

### 4.3.2 Chemical composition of essential oil of *E. saligna*

The percentage yields (v/w) of essential oil extraction indicate that *E. saligna* plant parts had leaves richer (0.38 ± 0.1%) in essential oils as compared to fruit (0.24 ± 0.09%) and bark (0.14 ± 0.04%). Table 4.2 shows the retention time (min), identified chemical constituents and percentage concentration of oils obtained from *E. saligna.* In the leaf essential oil, 16 compounds were identified, corresponding to 76.9% of the total essential oil composition. The leaf essential oil contained 50.2, 17.0, 9.7 and 1.3% monoterpenes hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes, respectively. The essential oil from leaves contained relatively high amounts of monoterpenes (67.2%) as compared to sesquiterpenes (9.7%) whereas diterpenes were in trace amounts. The major monoterpenes were; *p*-cymene (26.8%), α-pinene (14.7%), and borneol (4.7%) while sesquiterpenes were mainly, spathulenol (3.5%)., *iso*-leptospermone (3.2%) and α- guaiene (3.0%)

In the fruit essential oil, 35 compounds were identified corresponding to 84.9% of the total essential oil. The essential oil of leaves contained 39.7, 27.7, 10.6, 1.3, and 0.2 % monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and ditepenes, respectively. The major monoterpenes were sabinene (12.0%), terpin-4-ol (9.3%) andδ-3-carene (9.2%) was detected in fruit while the other hand, and sesquiterpenes were mainly italicene (2.4%) and epizonarene (1.5%) .

Seventeen (17) compounds accounting for 71.7% were identified in *E. saligna* bark essential oil composition. The bark essential oil contained relatively high amounts of sesquiterpenes (50.6%) as compared monoterpenes (14.8%) and diterpenes (11.9%). α-2-Carene (1.5%) and borneol and α-terpineol (5.1%), were the main monoterpenes in bark essential oil whereas sesquiterpenes were mainly trans-muurola-4 (14), 5-diene (13.2%) and *ar*-curcumene (7.4%) followed by *cis*-calamenene (6.2%) . Diterpenes were mainly flavesone (5.9%) and 13-*ep*-manool oxide (4.5%). Comparing the three essential oils, the highest amounts of monoterpenes were found in fruit (67.4%), followed by leaves (67.2%) and the lowest amount was in bark (14.7%). For sesquiterpenes, the highest percentage was detected in bark (50.6%) followed by fruit (10.6%) and the smallest amounts were in leaves (9.7%). Diterpenes were 11.9 % in bark and only present in small quantities in fruit and leaf essential oils.

The principal monoterpenes common in leaf, fruit and bark essential oils were α-terpineol and 1,8-cineole. The chemo types of *E. saligna* essential oil obtained from Busia were *p*-cymene (26.8%); sabinene (12.0%) and *trans*-muurola-4 (14), 5-diene (13.2%)

Table 4.2: Concentration (% v/w) and retention time (min.) of chemical constituents of *E. saligna* leaf, fruit and bark essential oils obtained from Busia

| RT (min) | Compound name | % Concentration | | |
|---|---|---|---|---|
| | | Leaves | Fruits | Bark |
| 9.65 | α-Thujene | - | 1.4 | - |
| 10.12 | α-Pinene | 14.7 | 5.9 | - |
| 10.43 | α-Fenchene | - | 1.4 | - |
| 10.72 | β-Pinene | 0.4 | - | - |
| 10.95 | Sabinene | - | 12.1 | - |
| 11.29 | Myrcene | - | 2.7 | - |
| 11.51 | *p*-Mentha-2,4(8)-diene | - | 2.1 | - |
| 11.64 | *p*-Cymene | 26.8 | 0.5 | - |
| 11.66 | δ-3-Carene | - | 9.2 | - |
| 11.71 | Eucalyptol | - | - | 1.1 |
| 11.73 | Sylvestrene | - | - | - |
| 11.80 | δ-2-Carene | - | - | 1.5 |
| 12.02 | Limonene | 1.7 | - | - |
| 12.10 | 1, 8-Cineole | 0.7 | 4.9 | 1.1 |
| 12.47 | *cis*- Linalool oxide | - | - | 0.9 |
| 12.55 | γ-Terpinene | 2.9 | 3.4 | - |
| 12.71 | *trans*-Sabinene hydrate (IPP vs OH) | - | 0.5 | - |
| 13.25 | Linalool | - | 3.1 | - |
| 13.52 | *endo*-Fenchol | 1.6 | - | - |
| 13.61 | Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene- | 2.3 | - | - |
| 13.72 | α-Campholenal | 2.3 | - | - |
| 13.75 | Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)- | - | 2.5 | - |
| 14.01 | Camphene | 1.4 | - | - |
| 14.08 | *p*-Mentha-1,5-dien-8-ol | - | 1.8 | - |
| 14.39 | Borneol | 4.8 | - | 5.1 |
| 14.51 | Umbullulone | - | - | 3.3 |
| 14.54 | Terpinen-4-ol | 1.6 | 9.3 | - |
| 14.75 | α-Terpineol | 6.0 | 3.1 | 5.1 |
| 14.78 | 2-Methylenebornane | - | 0.8 | - |
| 14.98 | Citronellol | - | 1.1 | - |
| 15.33 | Terpinolene | - | 1.0 | - |
| 15.85 | Bornyl acetate | - | 0.7 | - |
| 18.61 | *trans*-Muurola-4(14),5-diene | - | 1.3 | 13.2 |
| 18.68 | Epizonarene | - | - | 4.2 |
| 18.76 | α-Curcumene | - | - | 7.4 |
| 18.88 | β-Alaskene | - | 0.5 | - |

Table 4.2 cont'd

| | | % Concentration | | |
|---|---|---|---|---|
| **RT (min)** | **Compound name** | **Leaves** | **Fruits** | **Bark** |
| 19.01 | Epizonarene | - | 1.5 | 4.2 |
| 19.19 | Flavesone | - | - | 5.9 |
| 19.33 | *cis*-Calamenene | - | - | 6.2 |
| 19.77 | α-Guaiene | 3.0 | - | - |
| 19.77 | α-Cuprenene | - | 0.6 | - |
| 20.03 | Spathulenol | 3.5 | - | - |
| 20.31 | Italicene | - | 2.4 | - |
| 20.43 | *iso*-Leptospermone | 3.2 | - | 4.7 |
| 20.58 | Octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, 1.alpha.,4a.alpha.,8a.alpha.)- | - | 0.3 | - |
| 23.94 | Isophyllocladene | - | 0.8 | 1.2 |
| 24.52 | 13-*epi*-Manool oxide | - | - | 4.5 |
| 25.37 | Abietadiene | - | 0.5 | - |
| 25.81 | Nezukol | - | 0.2 | 1.5 |
| | **Total identified (%)** | **76.9** | **84.9** | **71.9** |
| | Monoterpenes (%) | 67.2 | 67.4 | 14.8 |
| | Sesquiterpenes (%) | 9.7 | 10.6 | 50.6 |
| | Diterpenes (%) | 0 | 0.2 | 11.9 |
| | Essential oil (%) yield(Mean± S.E) | [a]0.31 ±0.07 | 0.12 ± 0.02 | 0.09 0.04 |

Column used: HP-5MS

- = Absent

### 4.4 Discussion

The results of current study showed a clear intra-plant variation in C. *lusitanica* essential oil yield and chemical composition. The main constituents of all essential oils from *C. lusitanica* aerial parts were dominated by monoterpene hydrocarbons, which included α-pinene, β-phellandrene, β-pinene, terpinen-4-ol, δ-3-carene and p-cymen-8-ol. The results also reveal different chemo types depending on plant part. The chemo types of *C. lusitanica* essential oil obtained from Busia were: leaves, β-phellandrene; fruit and bark, α-pinene. The findings of this study are comparable to essential oil chemo types of *C. lusitanica* growing in Argentina containing α-pinene (11.2%), β-pinene (16.5%), δ-3-carene (19.4%), with other 27 compounds but no diterpenes ((Floreani *et al*., 1982). Carmo and Frazo (1989) also reported that *C. lusitanica* oil obtained from Portugal had similar components including α-pinene (18%), β-pinene and sabinene (13.2%) δ-3-carene and myrcene combined (8.2%) with other 17 compounds detected in trace amounts. However, the results are in contrast to essential oil compositions of *C. lusitanica* growing in Monteverde, Costa Rica which are dominated by α-pinene (40 to 82%), limonene (4 to 18%), isobornyl acetate (up to 10%) and *cis*-muurola-4 (14%), 5-diene (up to 7%) (Hassanzadeh *et al.*, 2010) and Cameroon, composed principally of umbellulone (17-18%) (Kuiate *et al*., 2006). The composition of the essential oil of *C. lusitanica* in the current study is also quite different from those reported from Brazil containing β-pinene, β-(Z)-ocimene, *endo*-fenchol and geraniol as major monoterpene constituents whereas the main sesquiterpenes are α-acoradiene, α-amorphene, thujopsan-2α-ol and 7α-*epi*-selinene. Similarly, the most abundant diterpenes are abietadiene and totarol (Filho *et al*., 2013).

It is worthwhile to note here that abietadiene, and *trans*-totarol were not detected in oils in current study. This is in contrast to the oil obtained from C. *lusitanica* growing in Portugal, which had relatively high concentrations of abietadiene (11-24%) and trans-totarol (5.1-6.5) (Adams, 1997) and Cameroonian *C. lusitanica* with germacrene D (18.5%) (Teke *et al*, 2013). Of interest also is the presence of β-phellandrene, γ-terpinene, (*E*-) caryophyllene, α-humulene, *p*-cymen-8-ol, *cis*-cadina-1(6), 4-diene and α-cedrene in high concentrations in current study as compared to data from other regions (Floreani *et al*., 1982; Kuiate *et al*., 2006; Hassanzadeh *et al.*, 2010, Teke *et al*., 2013). It is clear there are major differences between the chemical

composition of essential oils extracted from *C. lusitanica* in different regions and countries. A strong justification for this variation could be related to different climatic and edaphic conditions across the regions, which directly influence the metabolism of the plants (Chéraif *et al*., 2007), but also due to exposure to different biotic components (Brooker and Kleinig, 2006).

The chemical constituents of *E. saligna* essential oils reported in the current study reveals a clear intra-plant variation in yield and chemical composition. The main components of all essential oils obtained from aerial parts of *E. saligna* were dominated by α-pinene, *trans*-muurola-4 (14), 5-diene, sabinene, δ-3-carene and *ar*-curcumene. The results also reveal intra-plant variation chemo types. The chemo types of *E. saligna* essential oil obtained from Busia were identified as: leaves, α-pinene ; fruit, terpin-4-ol and bark, *trans*-muurola-4 (14), 5-diene.

In comparison, the results of this study have revealed percent chemical composition similar or higher than those of other researchers in different parts of the world. For instance, the major constituents of *E. saligna* growing in western highlands of Cameroon has been reported to contain α-pinene, *p*-cymene, 1,8 cineole, and terpinene (Tedonkeng *et al.,* 2004; Taponjou *et al.,* 2005; Dongmo *et al.,* 2008). In similar studies, Toloza *et al*. (2006) and Alzogaray *et al.* (2011) found *E saligna* growing in Argentina to contain a high percentage of 1,8-cineole and other compounds that included *p*-cymene, limonene, α-terpinene, γ-terpinene and α-terpineol. It is of interest to note here that *trans*-muurola-4(14), 5-diene, *ar*-macrocarpene, cryptone, flavesone, 2-undecanone and sabinene which were found in relatively high proportions(> 5%) in current study have not been reported elsewhere (Tedonkeng *et al.,* 2004; Taponjou *et al.,* 2005; Toloza *et al*., 2006; Dongmo *et al.,* 2008; Alzogaray *et al.,* 2011). Like in *C. lusitanica* above, there are many differences in the chemical composition of essential oils extracted from *E. saligna* in different regions and countries. A strong justification for this phenomenon could be related to different climatic and soil conditions between the regions and age of plants, which directly influence the metabolism of the plant and the exposure to different biotic components (Brooker and Kleinig, 2006; Chéraif *et al*., 2007).

It may also be concluded from this study that *C. lusitanica* and *E. saligna* essential oil chemical composition and classification into specific chemo types varies with plant part sampled. The

essential oils were rich in monoterpenes and their oxygenated derivatives known to have repellent and insecticidal (contact and fumigant) effects against insect pests of stored products. Essential oils rich in α-pinene, 1,8-cineole, γ-terpinene , terpinen-4-ol , *p*-cymene and eugenol most of which are present in *C. lusitanica* and *E. saligna* have been found to have repellent, fumigant and contact toxic effects against major pests of stored products (Liu *et al*., 2008; Ogendo *et al*., 2008b). The presence of these bioactive compounds in *C. lusitanica* and *E. saligna* provides hope for development of new natural insecticides that are ecologically benign and environmentally acceptable in other related applications.

# CHAPTER FIVE

# CONTACT TOXICITY OF LEAF ESSENTIAL OILS OF *Cupressus lusitanica* AND *Eucalyptus saligna* AGAINST *Tribolium castaneum, Acanthoscelides obtectus, Sitotroga cerealella* AND *Sitophilus zeamais*


## Abstract

In an effort to find eco-friendly alternatives to synthetic pesticides in grain storage, instant and residual contact toxicity of *C. lusitanica* and *E. saligna* leaf essential oils were evaluated against adult *T. castaneum, A. obtectus, S. cerealella* and *S. zeamais*. Bioassays were carried out under controlled conditions of temperature (28±2$^{o}$C) and relative humidity (65±5%) and 24h darkness. The experiments were laid out in a CRD with four replicates per concentration. In instant contact toxicity, test oil at five rates (0.00, 0.05, 0.10, 0.15 and 0.20% v/w) was applied on 10 g wheat and 20 g beans or maize in 100 ml glass jars. In residual contact toxicity each test oil was evaluated at the same rates as above but treated grain stored for 120 days. In the instant toxicity test *C. lusitanica* oil at 0.20% v/w caused 58.8, 77.6, 84.2 and 87.3% mortality of adult *S. zeamais, T. castaneum*, *S. cerealella* and *A. obtectus,* respectively, 168 h post-treatment.. Similarly, *E saligna* essential oil at same dose achieved a kill of 19.7, 56.3, 87.3 and 89.5% against adult *T. castaneum*, *S. zeamais, A. obtectus* and *S. cerealella,* respectively 168 h post-treatment. At at 0.20% v/w and 120 days storage, grains treated with *C. lusitanica* oils caused a mortality of 5.0, 17.5 and 65.0% against S. *zeamais, T. castaneum* and *A. obtectus*, respectively 168 h post-introduction of test insects. Similarly, E. *saligna* oils at above dose and 120 days storage caused a mortality of 5.0, 60.0 and 64.2.0% against adult *T. castaneum*, *S. zeamais* and *A. obtectus*, respectively 168 h post-introduction of test insects. *C. lusitanica* and *E. saligna* essential oils are potential contact insecticides for possible integration in stored product pest management.



**Key words**: contact toxicity, essential oil, $LC_{50,}$ mortality, residual toxicity


## 5.1 Introduction

The increasing serious problems of insect resistance to insecticides and the concomittant contamination of environment related due to the large-scale use of synthetic insecticides have directed the need for effective, biodegradable insecticides with greater selectivity (Campbell *et*

*al*., 2010; Obeng-Ofori, 2011, Ogendo *et al*., 2013). This awareness has created a worldwide interest in the scientific search for cost-effective, biodegradable and eco-friendly botanical insecticides to replace synthetic insecticides especially in smallholder agriculture. Among the natural products, plant essential oils and their constituents have attracted substantial scientific attention due to their phyto-toxic, repellent, anti-bacterial, herbicidal and antifungal effects (Batish *et al*., 2008; Liu *et al*., 2008). Plant essential oils are known for their potential to control storage insect pests and preserve food commodities. A number of essential oils and constituents have been classified as contact toxicants (Asawalam *et al*., 2006; Rosman *et al*., 2007, Ogendo *et al.*, 2011; Abay *et al*., 2012). Toxicants are specific types of chemicals, which directly kill insects. They are also referred to as insecticides.

However, few studies have reported the instant and contact residual toxicity of *C. lusitanica* and *E. saligna* against major stored product insects. Available information from literature indicate that *C. lusitanica* essential oil and constituents inhibit growth in *Enterococcus faecalis*, *Proteus mirabilis* and *Candida albicans* with minimum inhibition concentrations (MICs) of 1.25 and 0.16% for bacteria and fungi, respectively (Kuate *et al*., 2006). Similarly, Hassanzadeh *et al*. (2010) reported essential oils from the leaves of three different individuals of *C. lusitanica* to have antibacterial activity against *Bacillus cereus* and antifungal activity against *Aspergillus niger*.

Studies on the biological activity of Eucalyptus species extracts and constituents have revealed their promise as fumigants and contact toxicants (Jamaa *et al.*, 2013), repellents (Nivea *et al.*, 2013) against major pests of stored products. Furthermore, other studies have revealed their potential as antifungal (Dongmo *et al.,* 2008) and acaricidal (Tedonkeng *et al.*, 2004) agents. Moreover, Alzogaray *et al.* (2011) found *E. saligna* essential oils to be effective as fumigants and repellence against first instar of *Blattella germanica* L. and that there was a strong positive correlation between the fumigant activity of essential oils and their corresponding 1, 8-cineole and α-pinene concentration. Additionally, Taponjou *et al*. (2005) found essential oils extracted from *E. saligna* leaves to have toxic effects on *Sitophilus zeamais* ($LD_{50}$=0.36 $mlcm^{-2}$) and *Tribolium confusum* (0.48 $mlcm^{-2}$). Probit analysis showed that *T. confusum* was comparatively more susceptible ($LD_{50}$=0.96 $mlcm^{-2}$) to the toxic effect of cymol, a major constituent of *E.*

*saligna* oil than *S. zeamais* ($LD_{50}$=1.35 $mlcm^{-2}$). The insecticidal activity of eucalyptus oils has been associated with components such as 1, 8-cineole, citronellal, citronellol, citronellyl acetate, *p*-cymene, eucamalol, limonene, linalool, α-pinene, γ-terpinene, α-terpineol, alloocimene, and aromadendrene (Su *et al*., 2006; Batish *et al*., 2008; Liu *et al*., 2008). However, bioactivity and composition of essential oils varies with species, season, location, climate, soil type, and age of the plants, fertility regimeand the method of oil extraction (Brooker and Kleinig, 2006).

There is little information available on instant and residual contact toxicity of local aromatic plants including *C. lusitanica* and *E. saligna* against major coleopteran and lepidopteran pests of stored cereals and legumes. Considering the above prospects of essential oils as control agents of stored product insect pests, the current study purposed to evaluate instant contact and residual contact toxicity of essential oils obtained from leaves of *C. lusitanica* and *E. saligna* against *T. castaneum, A. obtectus, S. cerealella* and *S. zeamais*.

**5.2 Materials and methods**

The experimental conditions and methods on rearing of test insects and statistical data analysis are as described in section 3.1 and 3.2. Likewise, methods dealing with collection and preparations of plant materials, hydro-distillation of essential oils, analysis and identification of essential oil constituents are also described in section 4.2.

**5.2.1 Instant contact toxicity bioassay**

The instant toxicity of *C. lusitanica* and *E. saligna* leaf essential oils against adult *S. zeamais, S. cerealella, A. obtectus* and *T. castaneum*, were conducted according to Asawalam *et al*. (2006) and Ogendo *et al.* (2008b) with some modifications. Each test essential oil was applied to 10 g wheat and 20 g maize and bean grains in 100 ml glass jars at five concentrations (0.0, 0.05, 0.10, 0.15 and 0.20% v/w). The negative controls consisted of untreated grains whereas Actelic Super ™ (Primiphos-methyl + Permethrin) (0.056% v/w), and crude soya oil (1.0% v/w) served as positive controls. Grains were artificially infested each with 20 unsexed adults test insects. The experimental design and replicates were as described in Section 3.1. The numbers of dead insects were recorded 24, 72, 120 and 168 h post-treatment to estimate adult insect mortality.

The percentage adult mortality was computed according to Asawalam *et al*. (2006) and corrected for natural mortality using Abbott's formula (Abbott, 1925), respectively in equations 1 and 2

$$\text{Actual Mortality (\%)} = \frac{N_D}{N_T} X100 \quad (1)$$

$$\text{Corrected Mortality (\%)} = \frac{(P_O - P_C)}{(100 - P_C)} X100 \quad (2)$$

Where $P_O$ represent observed and $P_C$ control percent mortalities; $N_D$ and $N_T$ represent number of dead and total number of test insects per jar

**5.2.2 Residual toxicity bioassay**

Residual effects of test essential oils of *C. lusitanica* and *E. saligna* on adult *A. obtectus, S. zeamais* and *T. castaneum* were evaluated according to the method of Asawalam *et al*., (2006) with modifications. *S. cerealella* was not included in this bioassay because of insufficient insect numbers. The oils were applied to 50 g wheat (or 100 g beans or maize) grain samples in special self-sealing polythene bags (20 cm x 25 cm; 2L capacity) at rates of 0.0, 0.05, 0.10, 0.15 and 0.20% v/w. The negative control consisted of untreated grains whereas Actelic Super $^{TM}$ (0.056% v/w) and crude soya oil (1.0 % v/w) served as positive controls. The experimental design and replicates were as described in Section 3.1. The bags were then sealed and transferred to experimental room for long term storage (120 days). A random sub-sample (10 g wheat and 20 g bean grains) was then drawn from each experimental unit at 30, 60, 90 and 120 days after treatment. Into each sub-sample in 100 ml jars, 20 unsexed adult test insects ($N_T$) were introduced and the number of dead insects ($N_D$) recorded 24, 72, 120 and 168 h after treatment to estimate adult insect mortality. Actual and corrected percent mortalities in all contact toxicity studies were computed according to Asawalam *et al*. (2006) and Abbot (1925), respectively in equations 1 and 2 as in Section 5.2.1

## 5.3 Results

### 5.3.1 Instant contact toxicity

The results of the instant toxicity bioassay revealed that *C. lusitanica* and *E. saligna* leaf essential oil were toxic to adult *T. castaneum, A. obtectus, S. cerealella* and *S. zeamais*. The concentration of essential oil applied and time post-treatment significantly influenced the percentage adult mortality of all the test insects (ANOVA: $F_{(1, 9)} = 1.18\text{-}293$; $P< 0.05\text{- }0.001$). At 2.0 % v/w, *C. lusitanica* oil caused 84.2 %, and 86.0 % mortality of *S. cerealella* and *A. obtectus,* respectively 24 h post-treatment (Fig. 5.1a). *T. castaneum* and *S. zeamais* was more tolerant with mortalities of 18.2 and 59.2 % respectively 24 h post- treatment (Fig. 3a). Similarly, *E saligna* essential oil at 2.0 % v/w, achieved 86.9 % and 87.3 % mortality in *A. obtectus* and *S. cerealella*, respectively, 24 h post-treatment (Fig. 5.1b). On the other hand, at the same concentration, the mortality in *S. zeamais* and *T. castaneum* were rather low, 10.0 % and 11.8 % respectively 24 h after treatment.

*C. lusitanica* oil was highly toxic with $LC_{50}$ values of 0.05 and 0.11% v/w 24 h after contact for *S. cerealella* and *A. obtectus,* respectively (Table 5.1). On the hand, oil at the same concentration it was less toxic to *T. castaneum* and *S. zeamais* with $LC_{50}$ of 0.18 and 0.21 % v/w, respectively, 24 h post-treatment. *E. saligna* oil had similarly high toxicity levels with $LC_{50}$ values of 0.02 and 0.08 % v/w for *S. cerealella and A. obtectus*, respectively, 24 h post-treatment (Table 5.1). *T. castaneum* and *S. zeamais,* were more tolerant to *E. saligna* oil at the same concentration with $LC_{50}$ values of 0.19 and 17 % v/w, respectively, 24 h post- treatments.

At longer exposure period moderate mortalities of 77.6 % were observed with *C. lusitanica* oil against *T. castaneum* and 58 % against *S. zeamais* 168 h post-treatment (Fig. 5.2a). Similarly, moderate mortalities of 56.3 % were observed with *E. salgna* oil against *S. zeamais* and still low mortality of 19.7 % in *T. castaneum* 168 h post-treatment (Fig. 5.2b).

Toxicity levels increased in *C. lusitanica* oil against *T. castaneum* and *S. zeamais* with $LC_{50}$ of 0.11 and 0.13 % v/w, respectively, 168 h post-treatment (Table 5.2). *E. saligna* oil also became more toxic to *S. zeamais* 168 h post treatment recording a $LC_{50}$ value of 0.13 % v/w (Table 5.2). By comparison, all test insects were susceptible to the oils except *T. castaneum*. The positive controls, crude soya oil and Actelic super$^{TM}$ were toxic to test insects causing a mortality of 88.5 and 100 % mortality, respectively 72 h post-contact with treated grains

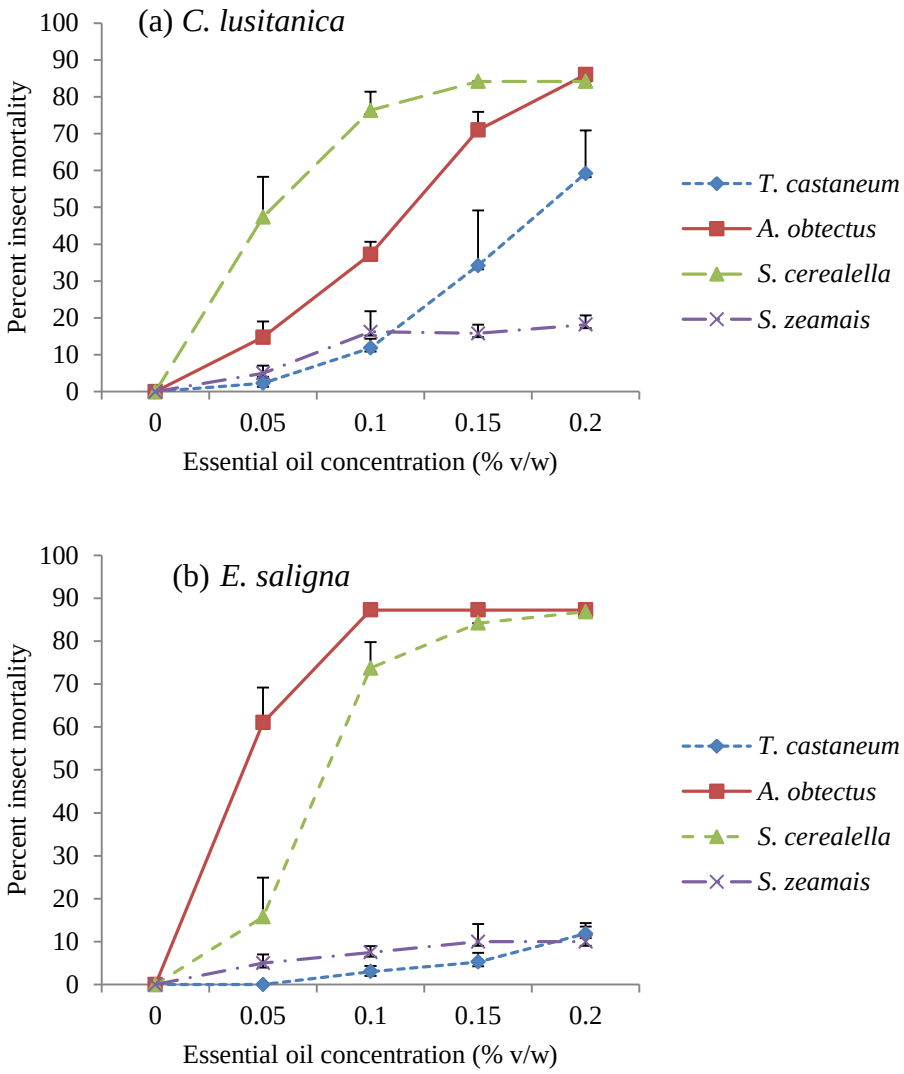



Fig.5.1: Percent mortality (Mean ± SE, n=4) of *T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais* after 24 h contact with five concentrations (v/w) of (a) *C. lusitanica* and (b) *E. saligna* leaf essential oils.

Table 5.1: $LC_{50}$ values (% v/w) of essential oils after 24-168 h of contact with four stored product insect pests (*T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais*)

| Plant EO/Insects | Contact Time (h) 24 | 72 | 120 | 168 |
|---|---|---|---|---|
| ***C. lusitanica*** | | | | |
| *T. castaneum* | 0.18(0.17,0.21[a])[c] | 0.17(0.15,0.18)[c] | 0.13(0.12,0.29)[c] | 0.12(0.11,0.14)[c] |
| *A. obtectus* | 0.11(0.17,0.21)[c] | 0.17(0.15,0.18)[c] | 0.13(0.12,0.13)[c] | 0.12(0.11,0.14)[c] |
| *S. cerealella* | 0.05(0.03,0.06)[c] | 0.02(0.01,0.04)[c] | 0.02(0.01,0.04)[c] | 0.02(0.01,0.04)[c] |
| *S. zeamais* | 1.21(0.46,25)[c] | 0.52(0.29,4.01)[c] | 0.19(0.16,0.26)[c] | 0.14(0.12,0.17)[c] |
| ***E. saligna*** | | | | |
| *T. castaneum* | 0.19(0.16,0.27) | 0.17(0.13,0.25) | 0.15(0.12,0.29)[c] | 0.11(-)[b] |
| *A. obtectus* | 0.02(-)[b] | 0.001(-)[b] | 0.001(-)[b] | 0.001(-)[b] |
| *S. cerealella* | 0.08(0.01,0.15) | 0.06(-)[b] | 0.04(-)[b] | 0.02(0.01,0.04)[c] |
| *S. zeamais* | 17(-)[b] | 0.39(0.27,0.91)[c] | 0.39(0.23,3.1)[c] | 0.13(0.10,0.17)[c] |

[a]Figures in parentheses represent the lower and upper 95 % confidence limits for the $LC_{50}$ values

[b]Signiificant response in Probit Regression Analysis at $P < 0.05$

[c]Insignificant response

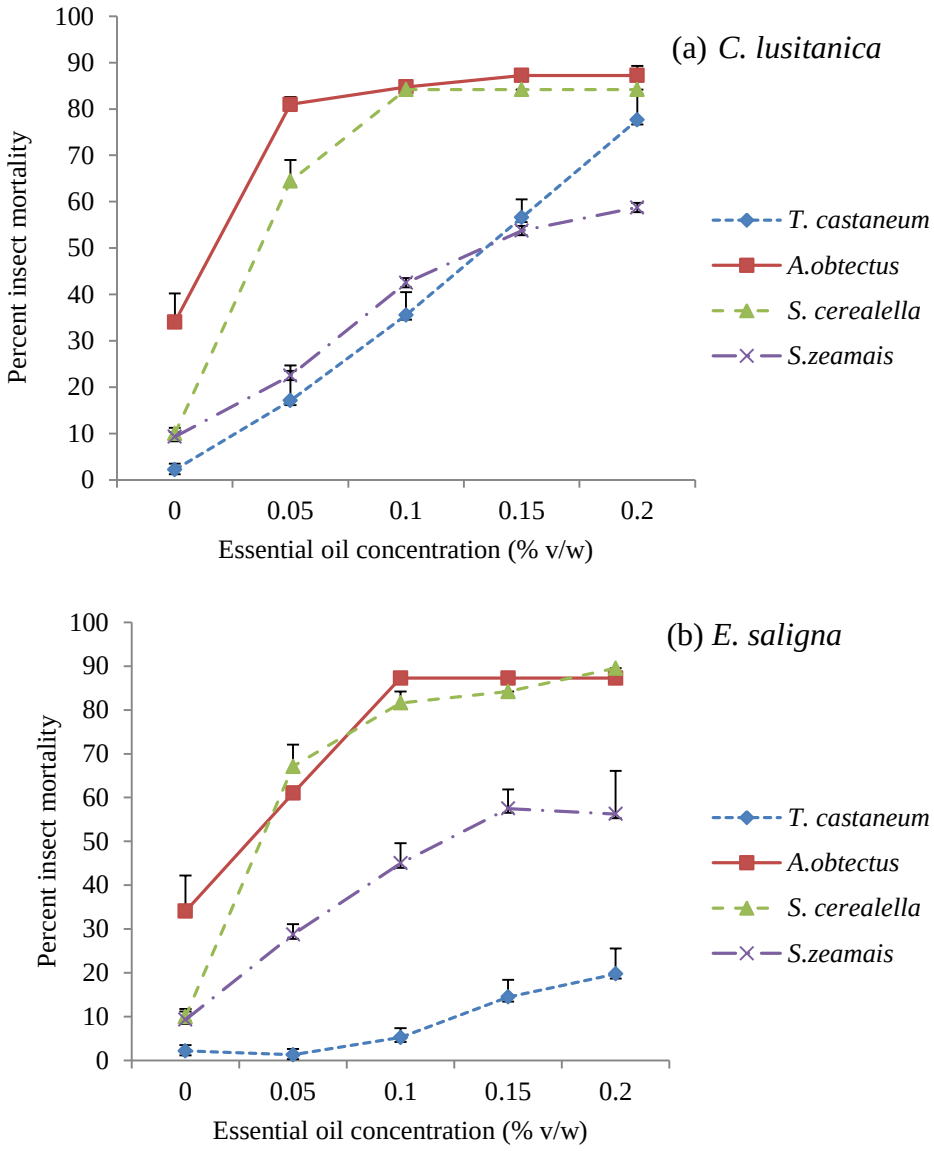



Fig. 5.2: Percent mortality (Mean ± SE, n=4) of *T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais* after 168 h contact with five concentrations (v/w) of (a) *C. lusitanica* and (b) *E. saligna* leaf essential oils.

### 5.3.2 Residual contact toxicity

The *C. lusitanica* leaf essential oils produced dose-, insect species- and storage duration-dependent residual contact toxicity against adult *T. castaneum* (ANOVA: $F_{(3, 9)} = 2.63\text{-}92.8$; $P< 0.05\text{- }0.001$), *A. obtectus* (ANOVA: $F_{(3, 9)} = 2.71\text{-}102.9$; $P< 0.001$) and *S. zeamais* (ANOVA: $F_{(3, 9)} = 3.83\text{-}76.5$; $P< 0.05\text{-}0.001$). At 0.20% v/w and treated grain storage period of 30 days, *C lusitanica* leaf essential oils caused 6.3, 25.0 and 85.0% kill of adult *T. castaneum*, *S. zeamais* and *A. obtectus*, respectively, 168 h post-introduction of test insects (Fig. 5.3a). The computation of $LC_{50}$ values revealed that *C. lusitanica* oil after a treated grain storage period of 30 days was toxic to adult *S. zeamais* and *A. obtectus* with $LC_{50}$ values of 0.07 and 0.12% v/w, respectively 168 h post introduction of test insects. On the other hand, the oil at the same concentration was less toxic to *T. castaneum* with $LC_{50}$ of 0.79, 168 h post-introduction of test insects (Table 5.2).

However, at the same concentration and 120 days grain storage duration *C lusitanica* oils caused a mortality of 5.0, 17.5 and 65.0% in adult *S. zeamais*, *T. castaneum* and *A. obtectus*, respectively 168 h post-introduction of test insects (Fig. 5.3 b). At the longest storage duration of 120 days, *C. lusitanica* oil was also toxic to *T. castaneum* and *A. obtectus* and *S. zeamais*, with $LC_{50}$ values of 0.12, 0.13 and 0.38% v/w, respectively 168 h post introduction of test insects (Table 5.2)

The *E. saligna* leaf essential oils produced dose-, insect species- and storage duration-dependent residual contact toxicity against adult *T. castaneum* (ANOVA: $F_{(3, 9)} = 3.66\text{-}90.73$; $P< 0.001$), *A. obtectus* (ANOVA: $F_{(3, 9)} = 4.65\text{-}189.4$; $P< 0.001$) and *S. zeamais* (ANOVA: $F_{(3, 9)} = 1.89\text{-}101$; $P< 0.05\text{-}0.001$). Results also indicated that at a dose of 0.20% v/w, *E. saligna* oil was highly efficacious over treated grain storage period of 30 days causing 32.5, 90.0 and 93.0% mortality against adult *T. castaneum*, *S. zeamais* and *A. obtectus*, respectively 168 h post-introduction of test insects (Fig. 5.4a). *E. saligna* oil treated grain storage period of 30 days had similarly high toxicity levels with $LC_{50}$ values of 0.003 and 0.005% v/w for *A. obtectus* and *S. zeamais* respectively 168 h post-introduction of test insects. *T. castaneum* was more tolerant, with $LC_{50}$ values of 0.51% v/w 168 h post-introduction of test insects (Table 5.3). The same results trend was observed at same concentration and 120 days grain storage duration where *S. zeamais* and *A. obtectus* were most susceptible to *E. saligna* oil causing mortalities of 90 and 93%, respectively

168 h post-introduction of test insects (Fig. 5.3a). However, at the same concentration and 120 days grain storage duration, *E. saligna* oils caused a mortality of 5.0, 60.0 and 64.2.0% in *T. castaneum*, *S. zeamais* and *A. obtectus*, respectively 168 h post-introduction of test insects. Similar $LC_{50}$ values were observed for *E. saligna* oil after 120 days grain storage duration with *T. castaneum, S. zeamais* and *A. obtectus* recording $LC_{50}$ values of 0.04, 0.10 and 0.70 % v/w respectively 168 h post-introduction of test insects (Table 5.3). By comparison, *A. obtectus* and was highly susceptible and *S. zeamais* moderately susceptible to *C lusitanica* oils whereas *T. castaneum* was the most tolerant of the three insect species tested. .

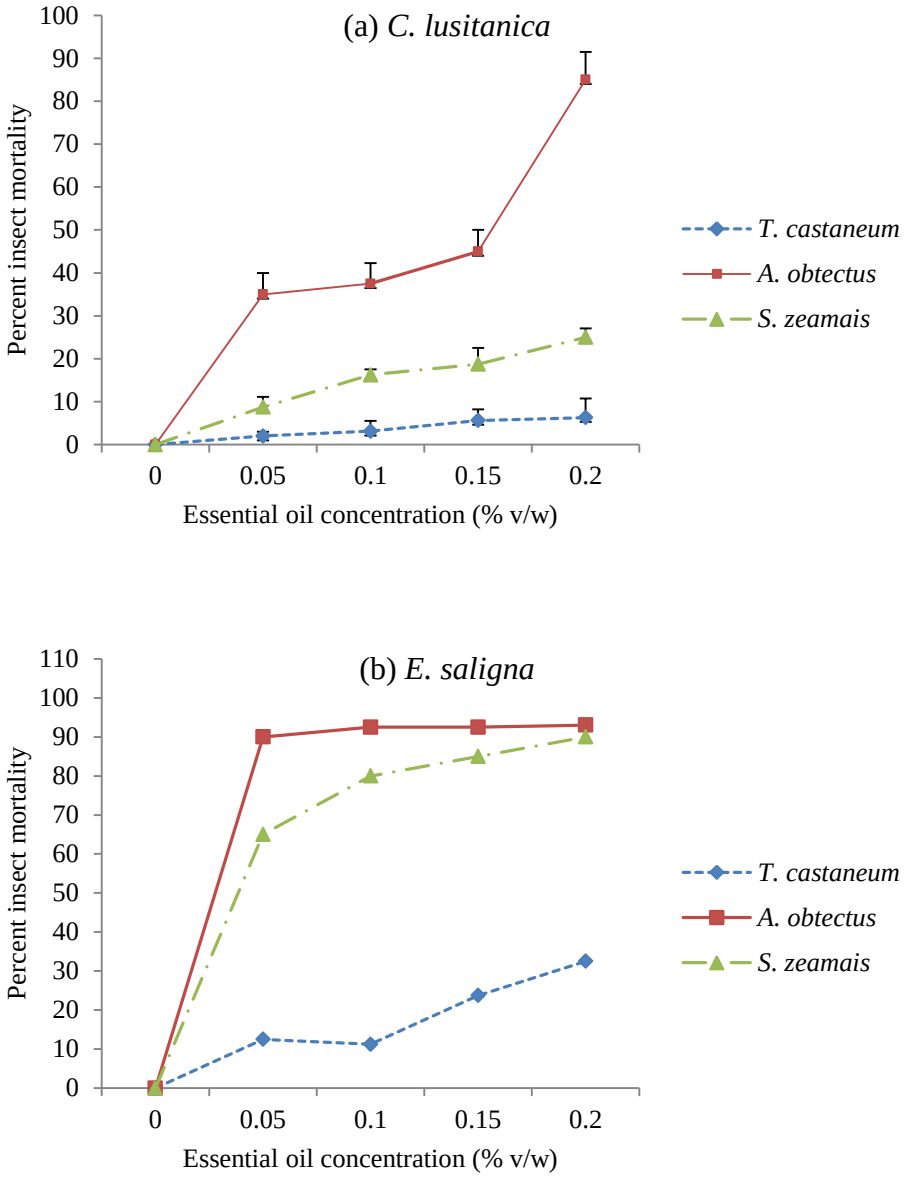



Fig.5.3: Percent mortality (Mean ± SE, n=4) of *T. castaneum*, *A. obtectus* and *S. zeamais* after 30 days contact with five concentrations (v/w) of (a) *C. lusitanica* and (b) *E. saligna* leaf essential oils.

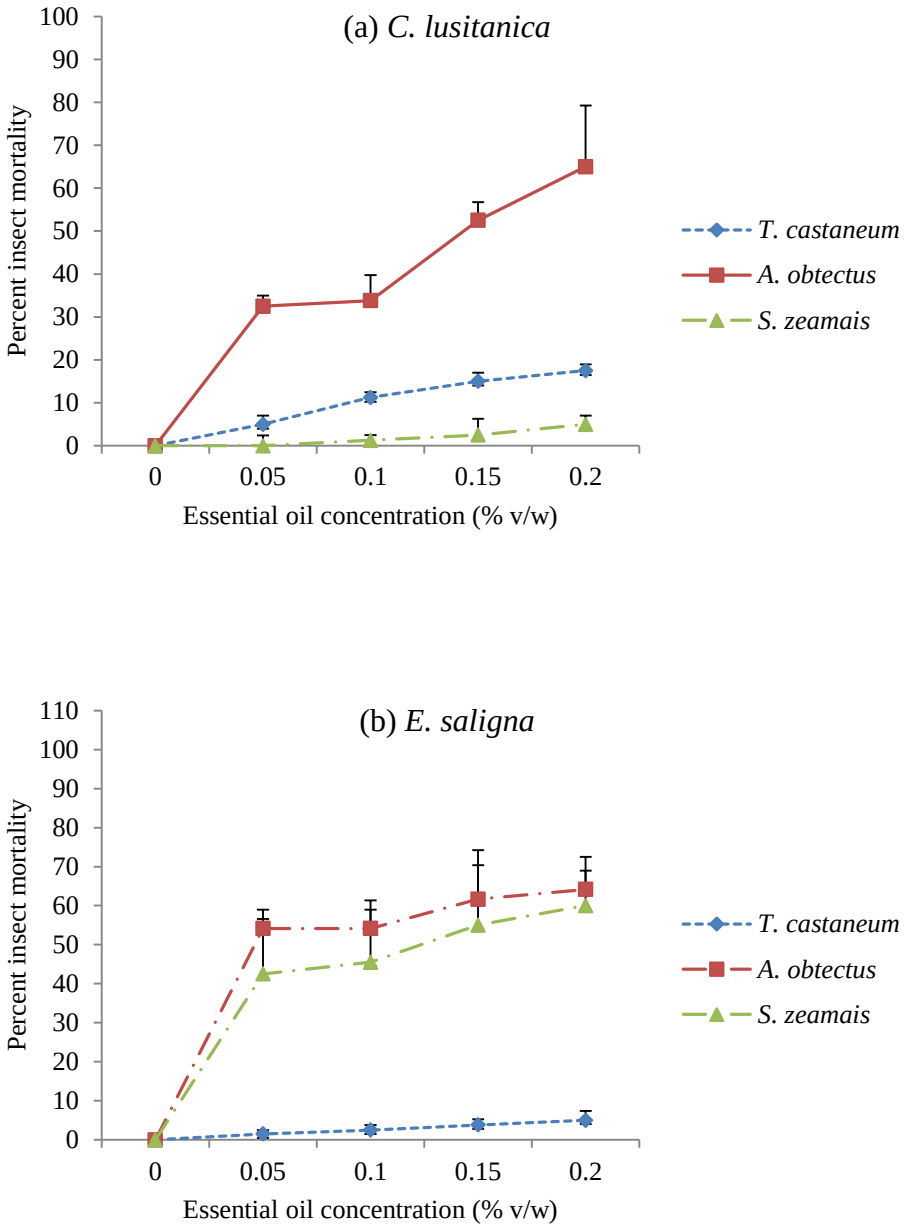



Fig. 5.4: Percent mortality (Mean ± SE, n=4) of *T. castaneum*, *A. obtectus* and *S. zeamais* after 120 days contact with five concentrations (v/w) of (a) *C. lusitanica* and (b) *E. saligna* leaf essential oils.

Table 5.2: $LC_{50}$ values (% v/w) of *C lusitanica* essential oils after 30-120 days contact with test insect pests (*T. castaneum, A. obtectus* and *S. zeamais*)

| [a]Insect/Time (h) | Grain Storage Duration(Days) 30 | 60 | 90 | 120 |
|---|---|---|---|---|
| ***T. castaneum*** | | | | |
| 24 | 0.29(0.20, 2.59[a]) | 22.5(-)[b] | 3.05(-)[b] | 0.78(-)[b] |
| 72 | 0.29(0.18, 6.51)[c] | 0.35(0.22, 14.1)[c] | 4.7(-)[b] | 3.22(-)[b] |
| 120 | 0.29(0.18, 6.51)[c] | 2.57(0.19,0.78)[c] | 0.18(0.12,1.50)[c] | 0.84(-)[b] |
| 168 | 0.79(-)[b] | 0.26(0.19,0.79)[c] | 0.18(0.12,1.51)[c] | 0.12(0.09,0.16)[c] |
| ***A. obtectus*** | | | | |
| 24 | 1.43(-)[b] | 1.22(0.14,2.60)[c] | 0.36(0.20,398.0)[c] | 0.49(0.23,8649)[c] |
| 72 | 0.44(0.28,1.40)[c] | 0.71(0.35,9.85)[c] | 0.61(-)[b] | 0.72(0.35,11.50)[c] |
| 120 | 0.19(-)[b] | 0.26(-)[b] | 0.19(-)[b] | 0.26(-)[b] |
| 168 | 0.12(-)[b] | 0.03(0.0, 0.051)[c] | 0.28(-)[b] | 0.13(-)[b] |
| ***S. zeamais*** | | | | |
| 24 | 1.72(-)[b] | 0.24(0.16,0.96)[c] | 0.35(0.23,114.7)[c] | 0.28(0.21,1.60)[c] |
| 72 | 0.29(0.19,1.50)[c] | 0.17(0.12,0.51)[c] | 0.31(0.20, 2.30)[c] | 0.41(0.24,63.10)[c] |
| 120 | 0.18(0.12,1.0)[c] | 0.07(0.04, 0.09)[c] | 0.30(0.21, 13.4)[c] | 0.24(0.15, 143.8)[c] |
| 168 | 0.07(0.04,0.10)[c] | 0.06(0.04, 0.07)[c] | 0.30(0.21, 13.4)[c] | 0.38(0.24,10.27)[c] |

[a]Figures in parentheses represent the lower and upper 95 % confidence limits for the $LC_{50}$ values

[b]Significant response in Probit Regression Analysis at $P < 0.05$

[c]Insignificant responses

Table 5.3: $LC_{50}$ values (% v/w) of *E. saligna* essential oils after 30-120 days contact with test insect pests (*T. castaneum, A. obtectus* and *S. zeamais*)

| Insect/Time (h) | Grain Storage Duration (Days) 30 | 60 | 90 | 120 |
|---|---|---|---|---|
| ***T. castaneum*** | | | | |
| 24 | 0.17(0.14,25.00[a])[c] | 0.36(-)[b] | 0.36(-)[b] | 0.36(-)[b] |
| 72 | 0.11(0.08, 0.13)[c] | 0.42(-)[b] | 0.42(-)[b] | 0.48(-)[b] |
| 120 | 0.106(-)[b] | 0.43(0.22,2126)[c] | 0.62(-)[b] | 0.73(-)[b] |
| 168 | 0.51(0.30, 2.21)[c] | 0.26(0.18,1.60)[c] | 0.62(-)[b] | 0.7(-)[b] |
| ***A. obtectus*** | | | | |
| 24 | 0.16(-)[b] | 0.22(0.19,0.42)[c] | 0.58(-)[b] | 0.58(-)[b] |
| 72 | 0.12(0.09,0.14)[c] | 0.16(-)[b] | 0.27(0.17, 3.90)[c] | 0.59(-)[b] |
| 120 | 0.06(0.05,0.08)[c] | 0.06(-)[b] | 0.12(0.09,0.17)[c] | 0.12(0.09,0.17)[c] |
| 168 | 0.003(-)[b] | 0.38(0.22, 4.77)[c] | 0.04(-)[b] | 0.04(-)[b] |
| ***S. zeamais*** | | | | |
| 24 | 0.21(-)[b] | 0.07(-)[b] | 0.09(-)[b] | 0.25(-)[b] |
| 72 | 0.20(0.16,0.33)[c] | 1.7(0.49,2687.7)[c] | 0.11(0.08, 0.15)[c] | 0.12(0.99,0.15)[c] |
| 120 | 1.79(0.49, 2688.7)[c] | 0.06(0.03,0.08)[c] | 0.06(0.03,0.08)[c] | 0.12(0.99,0.15)[c] |
| 168 | 0.005(-)[b] | 0.38(0.22, 4.77)[c] | 0.09(0.05,0.14)[c] | 0.10(0.05, 0.17)[c] |

[a]Figures in parentheses represent the lower and upper 95 % confidence limits for the $LC_{50}$ values

[b]Significant response in Probit Regression Analysis at $P < 0.05$

[c]Insignificant responses

**5.4 Discussion**

The results of this study have demonstrated that essential oils obtained from leaves of *C. lusitanica* and *E. saligna* are strong toxicants, an indication of the promise the two pesticidal plants hold in pest management. The fact that essential oils of *C. lusitanica* and *E. saligna*, at concentrations of 0.05-0.21% v/w were toxic enough to cause 50% kill of all test insects 24 h post treatment and high mortality 168 h post-treatment in all test insects except *T. castaneum,* offers hope for a practical solution to the insect pest menace. It is also manifested from the results that except *T. castaneum* the test insects were susceptible to *C. lusitanica* essential oils.

The results are in agreement with those of other researchers where several essential oils and constituents from plants in the various families have demonstrated strong dosage dependent contact toxicities against major coleopteran pests (Ogendo *et al.* 2008b; Ayvas *et al.* 2010, Nivea *et al.*, 2013). Ogendo *et al.* (2008b) found that apart from *T. castaneum,* essential oils from Lamiaceae, Verbenaceae and Fabaceae resulted in 90 - 100% kill of *C. chinensis, S. oryzae* and *R. dominica* 168 h post-treatment. Likewise Ayvas *et al.* (2010) reported essential oils of *Origanum onites* and *Satureja thymbra* to be highly effective against *Plodia interpunctella* and *Ephestia kuehniella*, with 100% mortality obtained after 24 h at 9 and 25 $\mu lL^{-1}$ for *P. interpunctella* and *E. kuehniella*, respectively. However, the insecticidal activity of the *Myrtus communis* oil was more pronounced than other oils tested against *A. obtectus* adults. However, among the tested insects, *A. obtectus* was the most tolerant species against the essential oils (Ayvas *et al.*, 2010). In contrast, *A. obtectus* was the most susceptible insect species in the current study. This could be attributed to differences in chemical constituents in the plants since the major compound found in oregano and savory was carvacrol whereas the main constituent of the myrtle was linalool while the major constituents of *C. lusitanica* in the current study were α-pinene, δ-3-carene, terpien-4-ol, and β-phellandrene.

There are few studies carried out on instant toxicity of essential oils of Eucalyptus species against coleopteran and lepidopteran pests of stored products to be compared to the results of current study. Available information indicate the $LC_{50}$ values of *Eucalyptus citriodora*, and *Eucalyptus staigeriana* oils against *C. maculatus* were in the range of 298.17 and 345.57 ppm in cowpea grains, respectively (Nivea *et al.*, 2013). As regards *E. saligna,* Taponjou *et al*. (2005)

found essential oils extracted from leaves to have toxic effects on *Sitophilus zeamais* ($LD_{50}$ = 0.36 $mlcm^{-2}$) and *Tribolium confusum* (0.48 $mlcm^{-2}$). The doses of 0.78 and 1.56 $mlcm^{-2}$of each oil was able to induce 100% mortality of insects within 5 days of exposure. They also found cymol a major constituent of oil to induce mortality of 71 and 100% against adult *S. zeamais* and *T. confusum*, respectively within 5 days of exposure at a dose of 1.30 $mlcm^{-2}$. Probit analysis showed that *T. confusum* was comparatively more susceptible ($LD_{50}$=0.96 $mlcm^{-2}$) to the toxic effect of cymol than *S. zeamais* ($LD_{50}$=1.35 $mlcm^{-2}$).

From the results of residual contact toxicity, *C. lusitanica* and *E. saligna* essential oils exhibited concentration- and storage and contact duration-dependent toxicity against *S. zeamais, T. castaneum* and *A. obtectus.* The fact that oils at a concentration of 0.20 v/w and storage duration of 30-120 days caused high mortalities of *S. zeamais* and *A. obtectus* demonstrates the potential of oils in the control of stored product insect pests during long term storage of products. However, *T. castaneum* was clearly tolerant to *C. lusitanica* and *E. saligna* essential oils for storage durations of 30-120 days at concentrations of 0. 20 v/w.

The same trend is observed in other studies where essential oils have exhibited different toxicities against coleopteran and lepidopteran insect pest of stored cereals and legumes. In short-term residual bioactivity studies with crude powders and extracts, significant adult insect mortalities and reproductive inhibitory effects against coleopteran pests of stored food commodities have also been reported (Al-Jabr, 2006; Ogendo *et al*., 2008a; Nivea *et al*., 2013 ). For instance, in local residual contact toxicity studies for 4-month storage duration, *T. vogelii* fruit essential oil had stronger residual toxicity (31- 47% kill) than fruit oil (18-21% kill) against *S. oryzae*. The converse was true for *O. americanum* leaf oil that caused 58-75% kill of *C. chinensis* compared to 37- 53% mortality rates by *T. vogelii* leaf oil (Ogendo *et al*., 2011). Nivea *et al.*(2013) reported that *O. americanum* essential oil was strongly toxic against *C. maculatus* adults ($LC_{50}$ = 0.23 $\mu lL^{-1}$ air) while the oils from *Hyptis suaveolens, H. spicigera* and *Lippia multiflora* exhibited higher $LC_{50}$ values of 1.30, 5.53 and 6.44 $\mu LL^{-1}$ air, respectively. The persistence of the biological activity of the four oils was variable and that from *O. americanum* was most persistent. In addition, Al-Jabr (2006) was able to demonstrate that complete mortality of *O. surinamensis* could be achieved by *Mentha viridis, Matricaria chamomilla* and

*Cinnamomum camphora camphora* at concentration more than 0.5%. Although, 1% of *Prunus amygdalus* and *Cymbopogon winterianus* gave complete mortality of *T. castaneum* after two weeks of exposure. Conversely, *Rosmarinus afficinalis* was the least toxic to both insect species.

The observed differential instant toxic effects of *C. lusitanica* and *E. saligna* essential oils against four coleopteran pests of stored food grains could be explained by individual and/ or synergistic bioactivity of major chemical constituents and differential responses by test insect species (Arriaga *et al*., 2005, Ogendo *et al.*, 2013). In the current study, it was clear that *A. obtectus* and *S. cerealella* were more susceptible to test essential oils as compared to *S. zeamais* and *T. castaneum.* The possible explanation for this variation is the fact that adult stages of *A. obtectus* and *S. cerealella* do not feed, hence become progressively weaker making them more susceptible to toxic effects of test oils. Although not directly investigated, the instant contact toxicity could also be attributed to α-pinene, δ-3-carene, terpien-4-ol, phellandrene, *cis*-cadina-1(6), 4-diene, α- cedrene and *trans*-muurola-4(14), 5-diene which were the major constituents of *C. lustanica* oil in the current study. Similarly, toxicity of *E. saligna* oil could be linked to its major compounds borneol, α- terpineol, α-pinene , *p*-cymene , α– guaiene, *iso*-leptospermone and spathulenol. Contact toxicity of essential oils against insect pests has been associated previously to presence of 1, 8-cineole, eugenol, methyl eugenol, and limonene and α-pinene among other bioactive essential oil constituents (Ilboudo *et al*., 2010; Abd-Elhady, 2012; Olivero-Verbel, *et al*., 2013.). The insecticidal activity of eucalyptus oils has been associated with components such as 1, 8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, a-pinene, g-terpinene, a-terpineol, alloocimene, and aromadendrene (Batish *et al*., 2006; Su *et al.*, 2006; Liu *et al*., 2008).

The mode of action of the essential oils could be due to contact toxicity through the insect cuticle, and fumigant toxicity through the respiratory and digestive systems. In addition, the toxic effect of essential oil constituents may be attributed to reversible competitive inhibition of acetylcholinesterase by occupation of the hydrophobic site of the enzyme's active center (Regnault-Roger *et al*., 2012). The current study has identified some possible botanical contact insecticides to replace synthetics currently in use. Moreover, provided with a proper formulation and dosage, the plant essential oils may be exploited for use against insect infestation at the

small scale farmer's level since they may be more effective and less cumbersome than application of dangerous synthetics.

# CHAPTER SIX

# FUMIGANT TOXICITY OF *Cupressus lusitanica* AND *Eucalyptus saligna* LEAF ESSENTIAL OILS AGAINST *Tribolium castaneum, Acanthoscelides obtectus, Sitotroga cerealella* AND *Sitophilus zeamais*.


## Abstract

*C. lusitanica* and *E. saligna* leaf essential oils were evaluated for fumigant potency against adult *T. castaneum, A. obtectus, S. zeamais* and *S. cereallela.* In the space fumigation test, each test essential oil was applied to filter papers (Whatman no. 1), suspended in the fumigation chamber and assayed at five doses (0, 5, 10, 15 and 20 µl$L^{-1}$ air whereas in grain fumigation the essential oil was assayed at 0, 30, 50 70 and 100 µl$L^{-1}$ air with exposure durations of 3, 5, 7 and 10 days. In the space fumigation assay, essential oil at 20 µl$L^{-1}$ air achieved 65.8 and 71.4 % mortality against adult *S. zeamais, T.castaneum, respectively* as compared to 100% mortality against adults of both *S. cerealella* and *A. obtectus* 168 h post-fumigation. *C. lusitanica* oil was toxic with $LC_{50}$ values of 3.71, 3.76, 13.54 and 15.28 µl$L^{-1}$ air against adult *A. obtectus, S. cerealella, S. zeamais* and *T. castaneum,* respectively 168 h post-fumigation. The *E. saligna* essential oil, at 20 µl$L^{-1}$ air, caused a mortality of 61.1, 92.1, 94.7 and 100% of adult *S. zeamais, T. castaneum A. obtectus* and S. *cerealella,* respectively, 24 h post-fumigation. The *E. saligna* leaf essential oil was also toxic with $LC_{50}$ values of 5.06, 6.71, 9.49 and 15.34 µl$L^{-1}$ air against *S. cerealella, A. obtectus, T. castaneum* and *S. zeamais,* respectively 168 h post-fumigation. In the grain fumigation studies, at 100 µl$L^{-1}$ air and 10 days grain fumigation duration, *C. lusitanica* leaf essential oil caused a mortality of 18.5, 28.8 and 100% against adult *T. castaneum, S. zeamais* and *A. obtectus,* respectively 168 h post-fumigation. The $LC_{50}$ values of *C. lusitanica* oil were 137.9, 38.7 and 2.3 µl$L^{-1}$ air against *T. castaneum, S. zeamais* and *A. obtectus,* respectively, after fumigation duration of 10 days and 168 h post-fumigation. Similarly, *E. saligna* oil at 100 µl$L^{-1}$ air and 10 days grain fumigation period caused mortalities of 31.3, 48.8 and 100% of adult S. *zeamais, T. castaneum, A. obtectus,* respectively 168 h post-fumigation. The $LC_{50}$ values for *E. saligna* oil in grain fumigation were 43.3 42.9 and 2.3 µl$L^{-1}$ air in *S. zeamais, T. castaneum* and *A. obtectus,* respectively. *C. lusitanica* and *E. saligna* essential oils are potential fumigants making them candidate botanical insecticides for possible incorporation in pest management technologies in small-scale agriculture.

**Key words**: Essential oil, grain fumigant, insect pest, $LC_{50}$, percent mortality, space fumigation

## 6.1 Introduction

Cereals and grain legumes are the major stable food crops in many African countries and hence pillars of food security (Obeng-Ofori, 2011). However, insect pest damage is responsible for about 20–50% of all food crop losses. The massive losses could attributed to favourable weather for optimum population increase of insect pests and traditional storage structures which expose grains to serious insect infestations among other factors (Nukenine, 2010). Currently *Sitophilus* spp., *S. cerealella, R. dominica, P. truncatus* and tenebrionid beetles on cereals, *A. obtectus* and *Callosobruchus* spp. on legumes rank high as major pests in storage. Stored grain pest control has relied on synthetic organochlorine and organophosphate chemicals in the form of dusts, granules, aerosols and fumigants among others. Fumigation is one of the most successful and cost effective methods used in the protection of stored cereals and legumes with minimal residues left on grains. Fumigants are chemicals, which at a required temperature and pressure, can exist in the gaseous state in sufficient concentration to be lethal to a given pest organism (Campbell *et al*., 2010). They may possess bactericidal, fungicidal, insecticidal and nematicidal properties (Batish *et al*., 2008). There are many chemical compounds that are volatile at normal temperatures and sufficiently toxic to act as fumigants (Suthisut *et al*., 2011). However most gases have been eliminated for use as commercial fumigants owing to unfavourable properties, the most important being chemical residues, health and environmental hazards (Obeng-Ofori, 2011, Regnault-Roger *et al*., 2012). Consequently, phosphine is the only remaining fumigant widely used on grains and other stored commodities after the phasing out of methyl bromide. Phosphine is carcinogenic and requires a long (44 days) exposure and high temperatures in sealed bins to achieve total insect control (Philips and Throne, 2010). Efforts are thus being made globally to replace these synthetic chemicals with botanical pesticides, which are natural in origin and biodegradable, have diverse physiological targets within insects, and consequently, may delay the evolution of insect resistance. One such natural pest control tactic for stored product pests is the use of essential oils.

Essential oils are composed of complex mixtures of monoterpenes, biogenetically related phenols, and sesquiterpenes obtained from plants through steam distillation (Athanassiou *et al*.,

2013). Many studies have demonstrated fumigant toxicity of plant essential oils to several species of stored product insects at different life stages (Toloza *et al*., 2006; Batish *et al*., 2008; Philips and Appel 2010**;** Suthisut *et al*., 2011; Nguemtchouin *et al*., 2013). Essential oils of the aromatic plants *Lavandula angustifolia, Rosmarinus officinalis, Thymus vulgaris* and *Laurus nobilis* were found by Rosman *et al* (2007) to have fumigant activity against adults of *S. oryzae*, *R. dominica* and *T. castaneum*. Essential oils of *Eucalyptus* and *Ocimum* species and *T. vogellii* have strong fumigant efficacy against *S. oryzae, R. dominica, C. chinesis* and *T. castaneum* (Ogendo *et al*., 2008b; Nivea *et al*., 2013; Jamaa *et al*., 2013). Similarly, fumigant toxicity of *Ageratum conzoides, Achillea fragrantissima* and *Tagetes minuta* (Gomah *et al*., 2015) and *Hyptis suaveolens, H. spicigera* and *Lippia multiflora (*Ilboudo *et al*., 2010) essential oils against *C. maculatus* has been documented.

Among the essential oil components, the monoterpenoids have drawn the greatest attention for fumigant activity against stored-product insects (Rajendran and Sriranjini, 2008; Philips and Appel, 2010). The high fumigant toxicity of linalool, linalyl acetate and 1.8-cineole was reported against the rice weevil *S. oryzae* and *R. dominica* (Rosman *et al*. 2007, Ogendo *et al* 2008b). Alzogaray *et al*. (2011) found essential oils from various Eucalyptus hybrids involving 8 species to be efficacious fumigants against *Blattella germanica* and efficacy was associated with α-pinene, 1, 8-cineole, *p*-cymene and γ-terpinene. Bachrouch *et al*. (2010) reported that *Pistacia lentiscus* essential oil was an effective fumigant against *Ephestia kuehniella* ($LC_{50}$ =1.84 $\mu lL^{-1}$ air), and *Ectomyelois ceratoniae* ($LC_{50}$=3.29 $\mu lL^{-1}$ air). The chemical constituents of *Pistacia lentiscus* essential oil included terpinen-4-ol (23.32%), α-terpineol (7.12%) and β-caryophyllene (22.62%) as major compounds. Nivea *et al*. (2013) reported essential oils of *Eucalyptus citriodora*, *E. staigeriana*, *Cymbopogon winterianus* and *Foeniculum vulgare* to be effective fumigants against *Callosobruchus maculatus* with $LC_{50s}$ values of 178.13- 345.57 ppm cowpea grains. The oils' main compounds were: *E.citriodora* (citronellal 89.59%; citronellyl acetate 3.34%; 1,8-cineole 2.87%), *E. staigeriana* (limonene 28.75%; geranial 15.20%; neral 12.16%), *C. winterianus* (geranial 21.83%; citronellal 10.94%) and *F. vulgare* (limonene 41.82%; (*E*)-anethole 17.91%; α-pinene 11.13%).

The reported fumigant activities prove that essential oils are sources of biologically active vapours that are potentially efficient insecticides. Therefore, the prospects of application of these natural fumigants in insects pest control options in stored products may be worthy of further research. In pursuit of this interest in essential oils as fumigants, the current study aimed at evaluating space and grain fumigant toxicity of of *C. lusitanica* and *E. saligna* leaf essential oils against adult *T. castaneum, A. obtectus, S. cerealella* and *S. zeamais*.

### 6.2 Materials and Methods

The experimental conditions and methods on rearing of test insects and statistical data analysis are as described in section 3.1 and 3.2. Likewise methods dealing with collection and preparations of plant materials hydro-distillation of essential oils, analysis and identification of essential oil constituents are also described in section 4.2

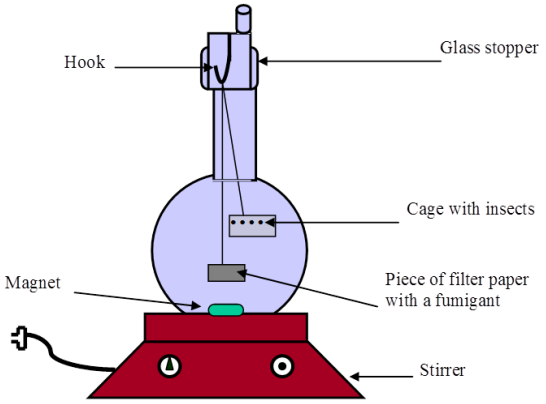
#### 6.2.1 Space fumigation bioassay

In the space fumigation assay, *C. lusitanica* and *E. saligna* leaf essential oils were evaluated against adult *S. cereallela, A. obtectus, S. zeamais* and *T. castaneum* in space fumigation chambers (Plate 6.1b) according to Ogendo *et al.* (2008b). Twenty unsexed adults ($N_T$) of each test insect species were introduced into meshed metallic cages with 5 g of food (wheat or bean or maize grains) and suspended from a hook in a 3.4 L flat-bottom glass flask space fumigation chamber. Each test essential oil was separately applied to provide dosages of 0, 5, 10, 15 and 20 $\mu l L^{-1}$ air on small pieces of Whatman No. 1 filter paper and then suspended in the chamber slightly below the cage. The treatments included untreated grains as negative controls. The experimental design and replicates were as described in section 3.1. A magnetic stirrer was used to ensure even distribution of fumigant in the chamber over a 24 h exposure period in experimental room. The numbers of dead ($N_D$) insects were recorded 24, 72, 120 and 168 h post- fumigation. The percentage adult mortality was computed according to Asawalam *et al*. (2006) and corrected for natural mortality using Abbott's formula (Abbott, 1925) respectively in equations 1 and 2 (section 5.2.1).

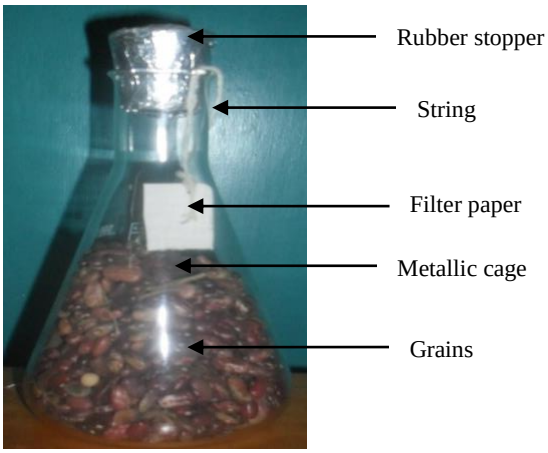
#### 6.2.2 Grain fumigation bioassay

In the grain fumigation test, essential oils extracted from *C. lusitanica* and *E. saligna* leaves were assayed in 600 ml glass chambers (Plate 6.1b) filled with 70% by volume with grain according to

Ogendo (2008b). Twenty ($N_T$) adults of *A. obtectus, S. zeamais* and *T. castaneum* were placed in metallic mesh and an aluminium lined rubber stopper at the other end. Cages were then introduced into the fumigation chamber and exposed for 3, 5, 7 and 10 days. The test oils were applied on small filter papers and suspended with a string in the fumigation chamber. Each test oil was assayed at five rates (0, 30, 50, 70 and 100 $\mu lL^{-1}$ air). The treatments included untreated as negative controls. The experimental design and replicates were as described in section 3.1. At the end of each exposure period, the glass stopper of each fumigation chamber was kept open for an hour for proper evacuation of fumigant. The numbers of dead (ND) adult insects were recorded 24, 72, 120 and 168 h post-treatment. The actual and corrected adult mortalities were computed as in section 5.2.1.




(a) Space fumigation




(b) Grain fumigation

Plate 6.1: Grain fumigation chambers (a) space fumigation and (b) grain fumigation

## 6.3 Results

### 6.3.1 Space fumigation

Instant fumigant toxicity of *C. lusitanica* and *E. saligna* leaf essential oils against four test insects resulted in significant essential oil concentration-, insect species- and fumigation duration-dependent insect mortality (ANOVA: $F_{(1, 9)} = 2.19\text{-}197.0$; $P< 0.05\text{-}0.001$). At 10 $\mu lL^{-1}$ air, *C. lusitanica* oil caused 90.6% mortality of both *S. cerealella* and *A. obtectus* 24 h post-fumigation (Fig. 6.1 a). The *E. saligna* leaf essential oil, at 15 $\mu lL^{-1}$ air, caused 94.7 and 100% kill for *A. obtectus* and S. *cerealella*, respectively, 24 h post-fumigation (Fig. 6.1 b). *C. lusitanica* oil was more toxic with $LC_{50}$ values of 4.08 and 4.71 $\mu lL^{-1}$ air against *A. obtectus* and *S. cerealella*, respectively 24 h post-fumigation. The *E. saligna* leaf essential oil was moderately toxic with $LC_{50}$ values of 6.71 and 7.02 $\mu lL^{-1}$ air for *S. cerealella* and *A. obtectus*, respectively 24 h post-fumigation.

*C. lusitanica* In comparison, the other two insect species were less susceptible with 65.8 and 71.4% mortality for *S. zeamais* and *T. castaneum*, respectively, 168 h post-fumigation with a higher concentration of 20 $\mu lL^{-1}$ air (Fig. 6.2 a). *E. saligna* The other two insect species were more tolerant, with 61.1 and 92.1% mortalities for *S. zeamais* and *T. castaneum* 168 h post-fumigation at 20 $\mu lL^{-1}$ air (Fig. 6.2 b). *C. lusitanica* at concentration of 20 $\mu lL^{-1}$ air was less toxic to *S. zeamais* and *T. castaneum* with $LC_{50}$ values of 13.54 and 15.28 $\mu lL^{-1}$ air, respectively 168 h post-fumigation (Table 6.1). *E. saligna* However, *T. castaneum* and *S. zeamais* were less toxic with $LC_{50}$ values of 9.49 and 15.34 $\mu lL^{-1}$ air, respectively 168 h post-fumigation.The cumulative percentage mortality of all insects tested was higher 168 h after treatment compared to 24 h (Figs. 2ab). *S. zeamais* and *T. castaneum* were tolerant to plant oils as compared to the other insect species tested.

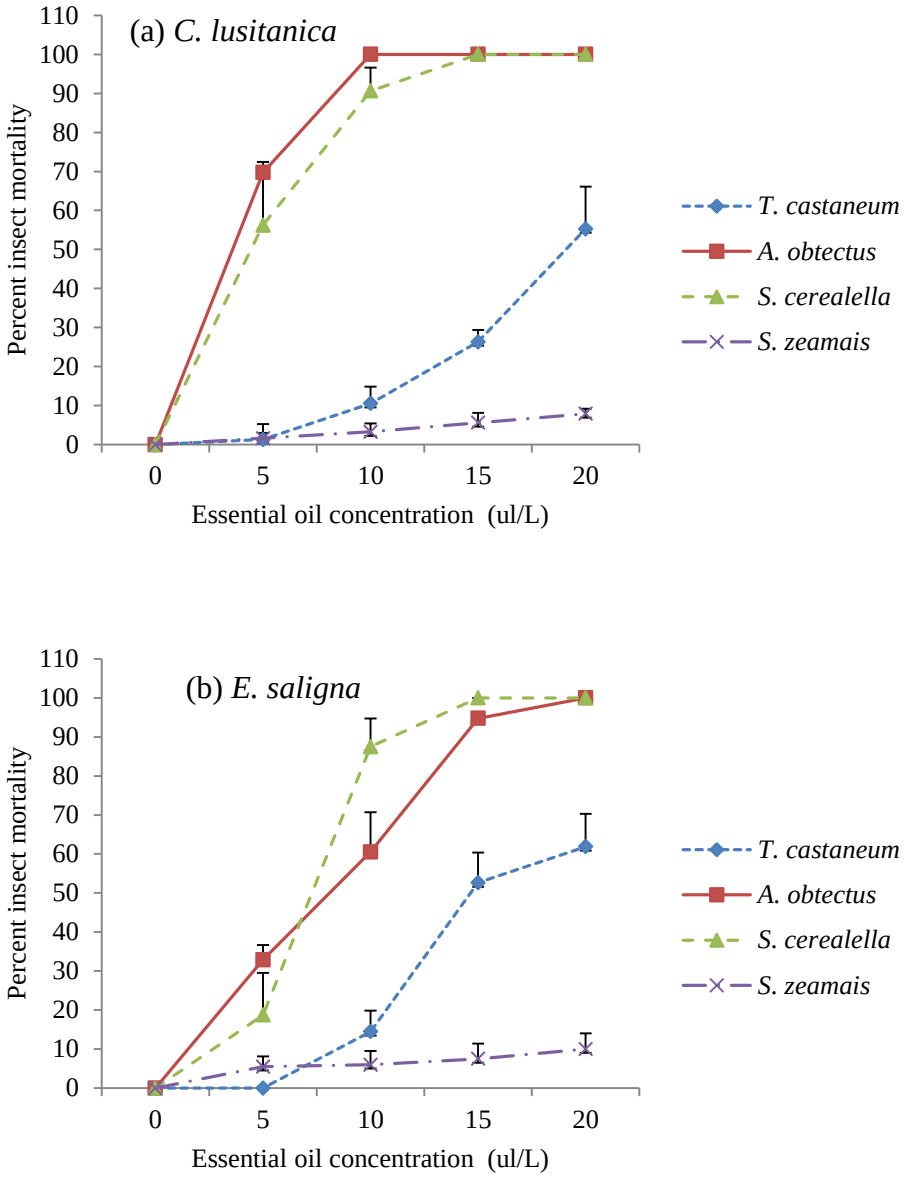



Fig.6.1: Percent mortality (Mean ± SE, n=4) of adult *T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais* after 24 h exposure to five concentrations of (a) *C. lusitanica* and (b) *E. saligna* leaf essential oils in space fumigation chambers.

Table 6.1: $LC_{50}$ values ($\mu lL^{-1}$ air) of essential oils against test insects (*T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais*) in space fumigation chambers 24 h post-fumigation.

| Plant EO/Insect[a] | Time (h) 24 | 72 | 120 | 168 |
|---|---|---|---|---|
| ***C. lusitanica*** | | | | |
| *T. castaneum* | 19.67(17.85,22.54[a])[c] | 19.02(17.03,22.13)[c] | 15.28(13.81,17.24)[c] | 15.28(9.86,78.49)[c] |
| *A. obtectus* | 4.08(3.23,4.77)[c] | 4.56(3.71,4.98)[c] | 3.61(2.00,4.25)[c] | 3.17(0.83,3.99)[c] |
| *S. cerealella* | 4.71(4.01,5.27)[c] | 3.69(2.36,4.29)[c] | 3.91(2.88,4.45)[c] | 3.76(2.55,4.34)[c] |
| *S. zeamais* | 29.11(18.11,1139)[c] | 20.84(-)[b] | 17.11(11.82,77.51)[c] | 13.54(-)[b] |
| ***E. saligna*** | | | | |
| *T. castaneum* | 16.09(11.96,30.47)[c] | 11.47(10.67,12.27)[c] | 10.79(8.12,13.30)[c] | 9.49(6.43,12.36)[c] |
| *A. obtectus* | 7.018(-)[b] | 5.37(-)[b] | 5.09(-)[b] | 5.06(-)[b] |
| *S. cerealella* | 6.71(6.25,7.48)[c] | 5.03(4.47,5.51)[c] | 4.54(3.65,4.87)[c] | 6.71(6.25,7.18)[c] |
| *S. zeamais* | 26.85(-)[b] | 30.79(23.03,55.58)[c] | 20.29(16.78,28.13)[c] | 15.34(-)[b] |

[a]Figures in parentheses represent the lower and upper 95 % confidence limits for the $LC_{50}$ values

[b]Significant response in Probit Regression Analysis at $P < 0.05$

[c]Insignificant responses

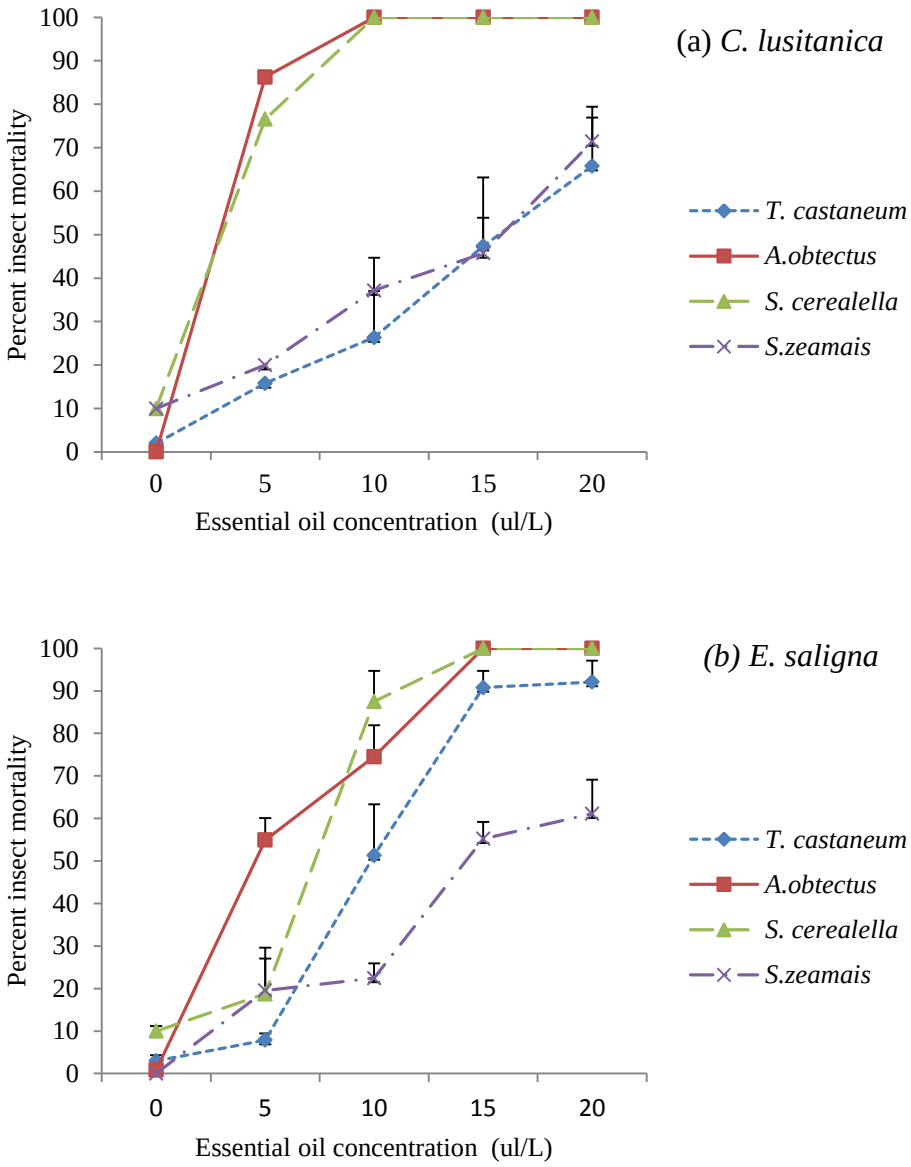



Fig.6.2: Percent mortality (Mean ± SE, n=4) of adult *T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais* after 168 h exposure to five concentrations of (a) *C. lusitanica* and (b) *E. saligna* leaf essential oils in space fumigation chambers.

### 6.3.2 Grain fumigation

Results of grain fumigant toxicity of *C. lusitanica* essential oils against *S. zeamais, T. castaneum* and *A. obtectus* after fumigation duration of 3-12 days are presented in Figs.6.3 & 6.4 and Table 6.2. Similarly, the fumigant toxicity of *E. saligna* leaf essential oils against *S. zeamais, T. castaneum* and *A. obtectus* after fumigation duration of 3-12 days are presented (Figs.6.3 & 6.4; Table 6.3). Fumigant toxicity of *E. saligna* oils against test insects resulted in significant essential oil concentration-, insect species- and fumigation duration-dependent insect mortality (ANOVA: $F_{(3, 27)} = 2.57\text{-}299.3$; $P< 0.01\text{- }0.001$).

Fumigant efficacy of *C. lusitanica* leaf essential oil was against test insects resulted in significant essential oil concentration-, insect species- and fumigation duration-dependent (ANOVA: $F_{(3, 9)} = 1.89\text{-}106.9$; $P< 0.05\text{- }0.001$). At 100 $\mu lL^{-1}$ air and 3 days grain fumigation duration, *C. lusitanica* oil caused 100% mortality of adult *A. obtectus* 168 h post-fumigation. At the same concentration and fumigation duration, the other two insect species were tolerant, with 18.8 and 22.5% mortalities for *T. castaneum* and *S. zeamais*, respectively 168 h post-fumigation at the same concentration of 100 $\mu lL^{-1}$ air (Fig. 6.3 a). The *E. saligna* essential oil, at 100 $\mu lL^{-1}$ air and grain fumigation duration of 3 days caused 100% kill for *A. obtectus* 168 h post-fumigation (Fig. 6.3 b). The other two insect species were less susceptible, with 27.5 and 35.0% mortalities for *T. castaneum* and *S. zeamais*, respectively 168 h post-fumigation with 100 $\mu lL^{-1}$ air (Fig. 6. 3 b). *C. lusitanica* oil was highly toxic with $LC_{50}$ values of 7.4 $\mu lL^{-1}$ air against *A. obtectus* after fumigation duration of 3 days and 168 h post-fumigation. However, the same test essential oil, was less toxic to *T. castaneum* and *S. zeamais* with $LC_{50}$ values of 195.5 and 115.2 $\mu lL^{-1}$ air respectively 168 h post-fumigation (Table 6.2). *E. saligna* oil at grain fumigation duration of 3 days treated grain had moderate toxicity levels with $LC_{50}$ values of 33.9 $\mu lL^{-1}$ air for *A. obtectus* 168 h post fumigation. *T. castaneum* and *S. zeamais* were more tolerant, with $LC_{50}$ values of 96.5 and 75.2 $\mu lL^{-1}$ air, respectively 168 h post-fumigation(Table 6.3).

Similarly, at the same concentration and 10 days grain fumigation duration *C. lusitanica* oils caused a mortality of 18.5, 28.8 and 100% in *T. castaneum, S. zeamais* and *A. obtectus*, respectively 168 h post-fumigation (Fig. 6.4 a). At 100 $\mu lL^{-1}$ air and 10 days grain fumigation duration, *E. saligna* oil caused 31.3, 48.8 and 100% mortality of adult *S. zeamais, T. castaneum* and *A. obtectus*, respectively 168 h post-fumigation (Fig. 6.4 b). The cumulative percentage mortality of all insects tested was higher 10 days fumigation duration after treatment compared

to 3 days. *A. obtectus* was most susceptible to plant oils as compared to the other insect species tested.

At the longest fumigation duration of 10 days, *C. lusitanica* oil was also toxic to *A. obtectus* with a $LC_{50}$ values of 2.3 $\mu lL^{-1}$ air 168 h post fumigation whereas same oil was moderately toxic to *S zeamais* with $LC_{50}$ values of 38.7 $\mu lL^{-1}$ air 168 h post fumigation. On the hand, *C. lusitanica* oil at the same concentration and fumigation period was less toxic to *T. castaneum* with $LC_{50}$ of 137.9 µL/L air 168 h post fumigation (Table 6.2). Higher toxicity levels were observed for *E. saligna* oil after 10 days grain fumigation duration with, *S. zeamais, T. castaneum* and *A. obtectus* recording $LC_{50}$ values of 43.3 42.9, and 2.3% $\mu lL^{-1}$ air respectively 168 h post fumigation (Table 6.3).

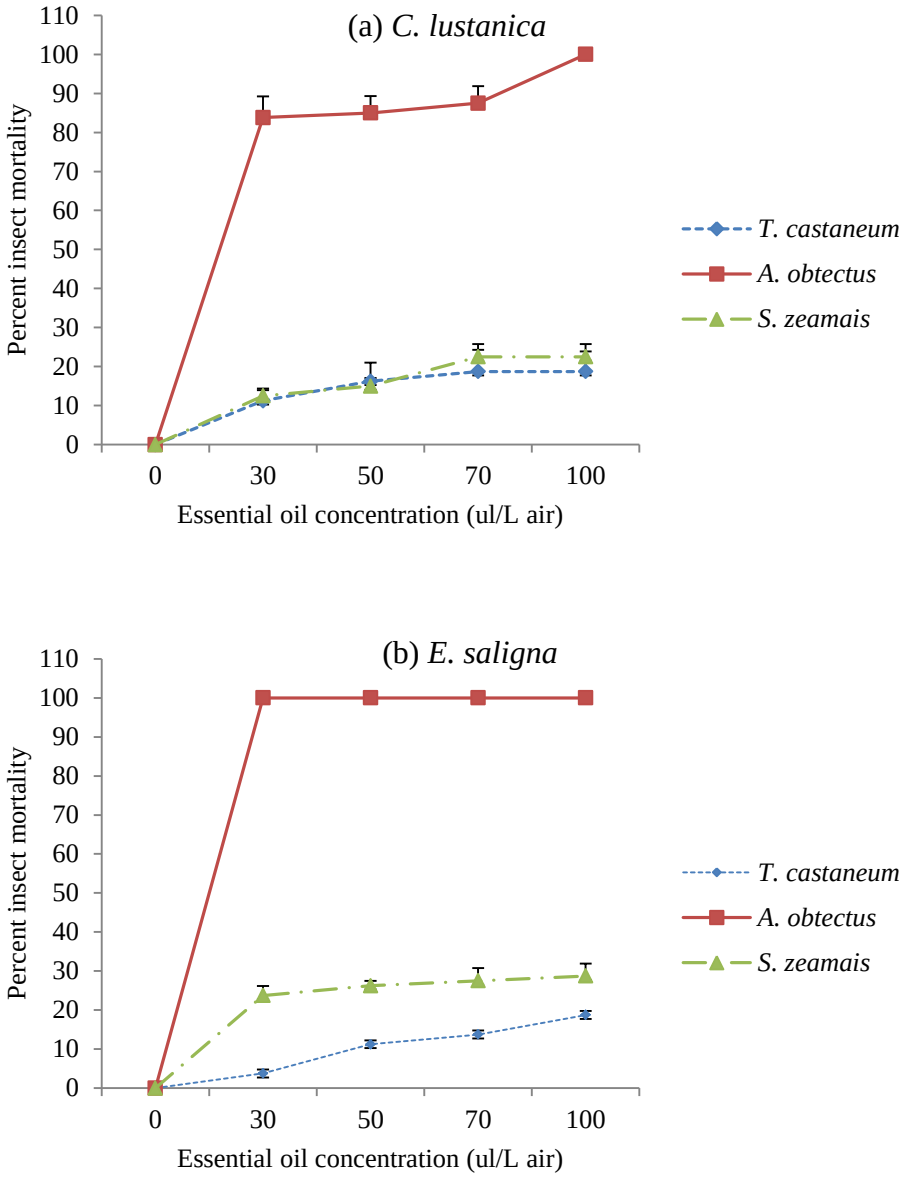



Fig. 6.3:Percent mortality (Mean ± SE, n=4) of *T. castaneum*, *A. obtectus* and *S. zeamais* after 3days grain fumigation with five concentrations of (a) *C lusitanica* and (b) *E. saligna* leaf essential oils.

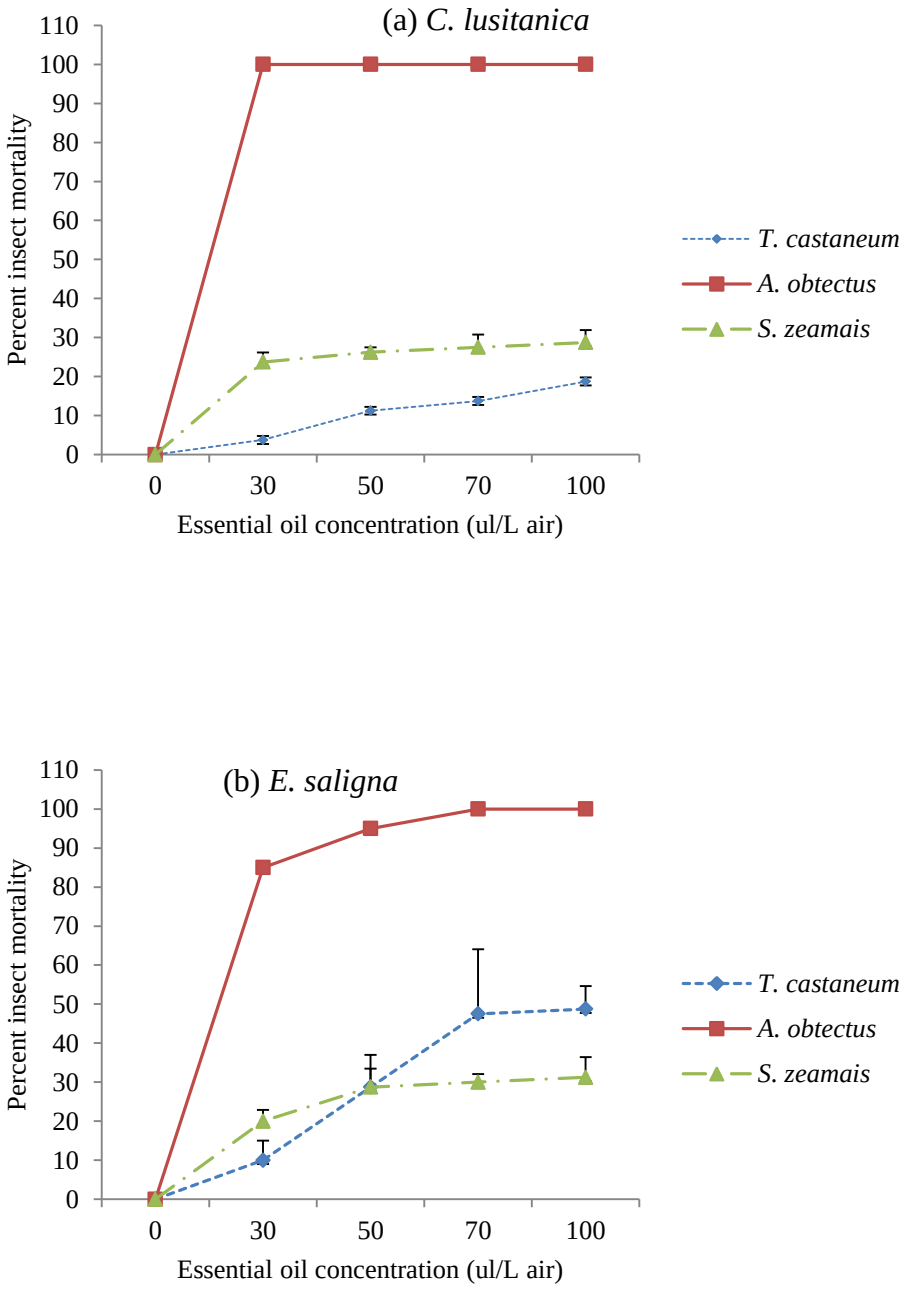



Fig.6.4: Percent mortality (Mean ± SE, n=4) of *T. castaneum*, *A. obtectus* and *S. zeamais* after 10 days grain fumigation with five concentrations of (a) *C lusitanica* and (b) *E. saligna* leaf essential oils.

Table 6.2: $LC_{50}$ values ($\mu lL^{-1}$ air) of *C. lusitanica* essential oils after 3-10 days grain fumigation against test insects (*T. castaneum*, *A. obtectus* and *S. zeamais*)

| | **Grain Fumigation Duration (Days)** | | | |
|---|---|---|---|---|
| **Insect/Time (h)** | **3** | **5** | **7** | **10** |
| *T. castaneum* | | | | |
| 24 | 360.4(-)[b] | 244.2(129.1, 17671.4[a]) | 255.1(140.0,6449.0)[cc] | 159.8(114.7, 412.9)[c] |
| 72 | 271.1(-)[b] | 288.6(126.0, 474008.0)[c] | 167.9(118.7,474.5)[c] | 123.9(101.3,194.6)[c] |
| 120 | 230.2(-)[b] | 191.2(108.8,9919.8)[c] | 147.8(107.8,348.9)[c] | 139.7(103.8,304.9)[c] |
| 168 | 195.9(-)[b] | 224.5(115.8,126606.8)[c] | 103.5(88.9,135.2)[c] | 137.9(98.5,382.2)[c] |
| *A. obtectus* | | | | |
| 24 | 68.4(60.7,79.1)[c] | 68.4(60.8,79.1)[c] | 2.3(-)[b] | 2.3(-)[b] |
| 72 | 2.3(-)[b] | 7.1(0.004,18.1)[c] | 2.3(-)[b] | 2.3(-)[b] |
| 120 | 7.1(0.004,18.1)[c] | 36.6(-)[b] | 2.3(-)[b] | 2.3(-)[b] |
| 168 | 7.4(0.0, 19.5)[c] | 2.3(-) | 2.3(-)[b] | 2.3(-)[b] |
| *S. zeamais* | | | | |
| 24 | 151.7(97.3,1279.0)[c] | 98.2(76.1,184.0)[c] | 164.3(111.6,593.4)[c] | 159.2(-)[b] |
| 72 | 139.9(93.3,646.9)[c] | 168.5(103.5,2157.7)[c] | 163.3(101.5,2009.1)[c] | 117.8(73.2,149.5)[c] |
| 120 | 130.0(87.2,806.5)[c] | 206.2(111.2,6884.4)[c] | 72.5(53.9,139.6)[c] | 53.1(25.2,90.2)[c] |
| 168 | 115.2(76.5,1945.2)[c] | 140(86.2,1322.8)[c] | 109.3(-)[b] | 38.7(-)[b] |

[a]Figures in parentheses represent the lower and upper 95 % confidence limits for the $LC_{50}$ values

[b]Significant response in Probit Regression Analysis at $P < 0.05$

[c]Insignificant responses

Table 6.3:$LC_{50}$ values ($\mu lL^{-1}$ air) of *E. saligna* essential oils after 3-10 days grain fumigation against test insects (*T. castaneum, A. obtectus* and *S. zeamais*)

| Insect/Time (h) | Grain Fumigation Duration (Days) 3 | 5 | 7 | 10 |
|---|---|---|---|---|
| *T. castaneum* | | | | |
| 24 | 218.9(-)[b] | 87.6(-)[b] | 91.1(-)[b] | 60.1(-)[b] |
| 72 | 245.1(140.2,3305.7[a])[c] | 99.6(80.3,154.8)[c] | 87.6(-)[b] | 56.1(49.6,63.8)[c] |
| 120 | 116.9(91.7,203.9)[c] | 99.6(80.3,154.8)[c] | 70.9(-)[b] | 49.9(44.0,55.8)[c] |
| 168 | 96.5(79.2,140.6)[c] | 38(19.9,50.6)[c] | 51.6(45.7,57.5)[c] | 42.9(38.8,46.9)[c] |
| *A. obtectus* | | | | |
| 24 | 39.6(37.7,41.5)[c] | 35.2(33.5,36.9)[c] | 22.4(16.8, 26.0)[c] | 16.8(8.6,21.9)[c] |
| 72 | 36.7(35.8,39.5)[c] | 33.9(32.1,35.8)[c] | 19.9(13.2,24.2)[c] | 12.4(3.2,19.0)[c] |
| 120 | 36.1(35.3,38.9)[c] | 33.2(30.1,36.2)[c] | 2.3(-)[b] | 2.3(-)[b] |
| 168 | 33.9(32.1,35.7)[c] | 32.4(30.7,34.3)[c] | 2.3(-)[b] | 2.3(-)[b] |
| *S. zeamais* | | | | |
| 24 | 304(136.9,29518.8)[c] | 132.0(96.3,322.3)[c] | 211.7(107.2,6310.0)[c] | 164.3(-)[b] |
| 72 | 171.5(98.9,2199.5)[c] | 106.6(81.3,215.2)[c] | 73.9(53.5,171.5)[c] | 66.3(-)[b] |
| 120 | 128.0(91.5,365.0)[c] | 106.6(81.3,215.2)[c] | 73.7(53.0,171.1)[c] | 66(-)[b] |
| 168 | 75.2(-)[b] | 63.1(50.4,83.8)[c] | 63.1(50.4,83.8)[c] | 43.3(8.0, 63.8)[c] |

[a]Figures in parentheses represent the lower and upper 95 % confidence limits for the $LC_{50}$ values

[b]Significant response in Probit Regression Analysis at $P < 0.05$

[c]Insignificant responses

## 6.4 Discussion

The results of space and grain fumigant bioassays showed that toxicity of *C. lusitanica* and *E. saligna* leaf essential oils varied with essential oil concentration applied, insect species and fumigation duration and post-fumigation time. Previous studies have reported intra- and inter-plant variations in fumigant toxicity of essential oils based on chemical compositions, pest susceptibility, degree of absorption of oil in treated commodity and route of entry of oil in target insects (Ogendo *et al*., 2008b; Rajendran and Sriranjini, 2008). The intra- and inter-plant variation in qualitative and quantitative chemical compositions of essential oils could be the cause of the differential responses by the test insect species (Arriaga *et al*., 2005, Regnault-Roger *et al*., 2012).

In the space fumigation, *C. lusitanica* oil and *E. saligna* leaf essential oils, at 10-15 $\mu lL^{-1}$ air, were effective fumigants of adult *S. cerealella* and *A. obtectus* 24 h post-fumigation. Adult *S. zeamais* and *T. castaneum* were more tolerant at low concentrations and post-fumigation time but became more susceptible at higher concentration of 20 $\mu lL^{-1}$ air and 168h post-fumigation time. The fact that plant essential oils were toxic at concentration of 100 $\mu lL^{-1}$ air and grain fumigation duration of 10 days against *A. obtectus* and *S. cereallela,* respectively 24 h post fumigation and 71.4 - 100% mortality in all test insects except *T. castaneum* 168h post fumigation proves that the plant oils have fumigant efficacy comparable to synthetic and other botanical pesticides. The recommended rate of phosphine is 8-12 $\mu gL^{-1}$, methyl bromide is 30-50 g $M^{-3}$ grain, 50 µl $L^{-1}$ air for the highly active Labiatae species oil, ZP51 and 50-150 mg $L^{-1}$ for allyl acetate to achieve 94.0-100% mortality of all insect pests of stored cereal and legume grains (Rajendran & Muralidharan 2005; Shaaya, *et al.*, 2006; Ogendo *et al.*, 2008b).

The results of this study are also comparable with the results of other researchers (Ogendo *et al*., 2008b; Ilboudo *et al*., 2010; Nguemtchouin *et al*., 2013). In local fumigation studies Ogendo (2008) was able to demonstrate that essential oils from plants in the family Lamiaceae, Verbenaceae and Fabaceae at a concentration of 50 $\mu lL^{-1}$ air, 7 days exposure and 120 days post-fumigation time was enough to obtain a mortality of 65.5% of *T. castaneum* and 95.4-100 % of *S. oryzae* and *R. dominica*. In previous studies Lee *et al*. (2005) reported $LT_{50}$ values of 16.2, 17.4 and 9.1 h were for *E. blakelyi*, *Melaleuca fulgens* and 1, 8-cineole, respectively, against

adult *S. oryzae*. Ilboudo *et al*. (2010) was able to demonstrate that essential oils extracted from *Ocimum americanum* to be very toxic towards *C. maculatus* adults ($LC_{50}$ = 0.23 $\mu lL^{-1}$ air) while the oils from *Hyptis suaveolens*, *H. spicigera* and *Lippia multiflora* exhibited higher $LC_{50}$ values of 1.30 ; 5.53 and 6.44 $\mu lL^{-1}$ air, respectively. In unrelated studies, Alzogaray *et al.* (2011) found that essential oils from hybrids involving 8 Eucalyptus species were efficacious fumigants against *Blattella germanica* L with lowest knockdown time (min) 50% ($KT_{50}$) in the range of 57.9-74.5. In a study of fumigant toxicity of E. *camaldulensis* and *E. leucoxylon* against adults and last instars larvae of the carob moth *Ectomyelois ceratonia* with *E. camaldulensis* essential oil totally effective(100% mortality), while for *E. leucoxylon* oil, 94.5% and 98.4% mortally were obtained, respectively, after 3 and 7 days of exposure (Jamaa *et al*., 2013).

There are numerous reports on the insecticidal activity of the essential oils constituents such as carvacrol, thymol, γ-terpinen and terpinen-4-ol (Lee *et al*., 2003; Rosman *et al*., 2007; Nivea *et al*., 2013). Despite not being tested directly, the activity of the essential oils in the current study may be attributed to major constituents of *C. lustanica* oil such as α-pinene, δ-3-carene, terpien-4-ol, phellandrene, *cis*-cadina-1(6), 4-diene, α- cedrene and *trans*-muurola-4(14), 5-diene. Similarly, fumigant toxicity of *E. saligna* oil could be linked to its major compounds like borneol, α- terpineol, α-pinene , *p*-cymene , α- guaiene, *iso*-leptospermone and spathulenol. Lee *et al*. (2003) proved that *Tribolium castaneum* could be controlled by 1, 8-cineole, 1-fechone, linalool and pulegone, and recommended monoterpenes as suitable fumigants because of high volatility, fumigant efficacy and safety. Similarly, four essential oils constituents at 0.1 µl/720 ml volume, eugenol, 1,8-cineole, camphor and linalool caused 85-100, 80-100 and 0-13% mortality of adult *S. oryzae*, *R. dominica* and *T. castaneum*, respectively, 24 h after treatment (Rosman *et al*., 2007). Alzogaray *et al*.(2011) found essential oils constituents such as α-pinene, 1, 8-cineole, *p*-cymene and γ-terpinene to have knockdown ($KT_{50}$) values of 55.3-178.3 minutes against *Blattella germanica*. In a study of fumigant toxicity, Bachrouch *et al*. (2010) found *Pistacia lentiscus* essential oil to be effective fumigants against *Ephestia kuehniella* ($LC_{50}$=1.84 $\mu ll^{-1}$ air), and *Ectomyelois ceratoniae* ($LC_{50}$=3.29 $\mu lL^{-1}$ air). The chemical constituents of *Pistacia lentiscus* essential oil included terpinene-4-ol (23.32%), α-terpineol (7.12%) and β-caryophyllene (22.62%) as major compounds. Nivea *et al*. (2013) reported essential oils of *Eucalyptus citriodora*, *E. staigeriana*, *Cymbopogon winterianus* and *Foeniculum vulgare* to be effective

fumigants against *Callosobruchus maculatus* with $LC_{50s}$ of 178.13- 345.57 ppm cowpea grains. The oils' main compounds were: *E. citriodora* (citronellal- 89.59%; citronellyl acetate- 3.34% and 1,8-cineole- 2.87%), *E. staigeriana* (limonene- 28.75%; geranial- 15.20% and neral- 12.16%), *C. winterianus* (geranial- 21.83%; citronellal- 10.94%) and *F. vulgare* (limonene- 41.82%; (*E*)-anethole- 17.91% and α-pinene- 11.13%).

The observed strong fumigant toxicity could be attributed to individual and/or blend effects of bioactive chemical constituents contained in the essential oil. The moderate to strong fumigant toxicities of the two essential oils could possibly be due to their differential compound structure-activity relationships and inter-insect species' responses as manifested in physiological-structural induced cellular changes resulting in poisoning of insects by blocking octopamine receptors (Priestley *et al*., 2006). Lee *et al*., (2003) proved there was contact toxicity through the insect cuticle, and fumigant toxicity through the respiratory and digestive systems. Several reports also indicate that monoterpenoids cause insect mortality by inhibiting acetyl cholinesterase enzyme (AChE) activity (Regnault-Roger *et al*., 2012). Apart from the above , *A. obtectus* and *S. cerealella* were more susceptible to test essential oils as compared to *S. zeamais* and *T. castaneum* possibly due to the fact that adult stages of *A. obtectus* and *S. cerealella* do not feed, hence become progressively weaker making them more susceptible to toxic effects of test oils.These results, and those reported earlier, also indicate that the insecticidal activity of the essential oils varies depending on the stage of the insect, the species and the plant origin of the essential oil (Negahban *et al*., 2006).

It is evident from the results of current study that essential oils are promising fumigant alternatives to synthetic insecticides for controlling coleopteran and lepidopteran pests of stored products. If the cost-effective commercial production and regulatory barriers are solved, the essential oils obtained from these plants can effectively be used as part of integrated pest management strategies.

# CHAPTER SEVEN

# REPELLENCE OF *Cupressus lusitanica* AND *Eucalyptus saligna* LEAF ESSENTIAL OILS AGAINST FOUR MAJOR INSECT PESTS OF STORED GRAINS


## Abstract

Laboratory bioassays were conducted to evaluate the repellent efficacy of C. *lusitanica* and *E. saligna* leaf essential oils against adult *T. castaneum, A. obtectus, S. cerealella* and *S. zeamais.* In instant repellency, each test essential oil was evaluated at five concentrations (0.00, 0.05, 0.10, 0.15 and 0.20% v/w) in an alternate untreated (control)-treated choice bioassay system whereas in residual repellence, oils were assayed at above rates but treated grain samples were stored for 30, 60, 90 and 120 days. DEET was used as a positive control. In instant repellence test *C. lusitanica* leaf essential oil elicited strong to very strong repellence against *T. castaneum* with percentage repellence (PR) values of 65-92.5% but weakly repellent against the other test insects with PR values less than 30%, 24 h post- exposure. The PR values for *E. saligna* leaf essential oil, at 0.20 % v/w, against *T. castaneum, A. obtectus,* and *S. cerealella* were 9.3, 4.0 and 1.8, respectively 24 h post- exposure. However, both *C. lusitanica* and *E. saligna* leaf essential oils produced decreasing PR values of 12– -4%, 55.5–1.8% and 38.9– -10% against *A. obtectus, S. cerealella* and *S. zeamais*, respectively 24 post-treatment. In residual repellence bioassay, *C. lusitanica* leaf essential oil, at 0.20% v/w and 120 days grain storage duration, was moderately repellent with PR values of 37.9, 47.6 and 51.1% against adult *A. obtectus, S. zeamais* and *T. castaneum,* respectively 12h post-introduction of test insects. In *E. saligna* leaf essential oil, at the same concentration and 120 days grain storage duration; moderate repellence was recorded with a PR value of 52.4% in *T. castaneum* but weakly repellent to *A. obtectus* (34.0%) and *S. zeamais* (36.6%), 12 h post-introduction of test insects. Results point to *C. lusitanica* and *E. saligna* essential oils as promising natural repellents of stored product insect pests for possible inclusion in insect pest management options.



**Key words:** choice bioassay, DEET, essential oil, percent repellence, stored product.

## 7.1 Introduction

Insect pests cause 5-10% and 20-30% damage to stored grains in the temperate and tropical countries, respectively (Philips and Throne, 2010). In this scenario, protection of stored grains against insect infestation is an urgent matter. Common tools available for managing stored product insect pests include synthetic contact insecticides and fumigants, biological control agents as well as appropriate modified atmospheres through metal silos and hermetic storage technology. However, human and environmental health risks associated with the use of synthetic insecticides have led to advocacy for natural, safer and sustainable alternatives in pest control. Naturally occurring botanical insecticides, which have traditionally been used to kill insects may provide this option (Abate *et al*., 2007; Deng *et al*., 2009; Ogendo *et al*., 2012). Essential oils obtained from plants are in particular under investigation for their broad-spectrum pest control properties (Regnault-Roger *et al.,* 2012).

Several studies on pesticidal potency of plant essential oils and their constituents have been demonstrated to have repellent (Nerio *et al*.,2010; Alzogaray *et al*., 2011; Bett *et al*., 2013), antifeedant (Wambua *et al*., 2011) and reproduction inhibition properties (Regnault-Roger *et al.*, 2012; Tucker *et al*., 2014) against several insect pests of stored food grains. A repellent may be considered as a compound applied to skin, clothing, stored product or other substrates that decreases normal contact time of arthropods with the treated surface. Essential oils are volatile mixtures of hydrocarbons with a diversity of functional groups, and their repellent activity has been linked to the presence of monoterpenes and sesquiterpenes. However, in some cases, these chemicals can work synergistically, improving their effectiveness (Nerio *et al*, 2010). The basils (*Ocimum* spp.), lemon grass (*Cymbopogon* spp) and *Eucalyptus* spp. are among some plant families with promising essential oils used as repellents (Nerio *et al*, 2010).

The repellent ability of essential oils and constituents from these plant species has already been reported (Nerio *et al*., 2010; Ogendo *et al*., 2012, Regnault-Roger *et al*., 2012). Ogendo(2008) demonstrated that essential oils and constituents obtained from *L. camara*, *O. americanum*, and *T. vogelii* were effective repellents against *S. oryzae*, *T. castaneum*, *C. chinensis* and *R. dominica.* In other related studies Liang *et al.* (2013) showed that the essential oils of *Curcuma*

*longa*, *Epimedium pubescens*, *Lindera aggregate*, *Nardostachys chinensis*, *Schizonepeta, tenuifolia, Zanthoxylum schinifolium*, and *Z. officinale* exhibited strong repellent action against *T. castaneum*. The repellent action of the different essential oils against *T. castaneum* were reported to decrease in the order of *Cymbopogon martini, C. flexuosus* and *Lippia origanoides* (Caballero-Gallardo *et al*., 2012)

The highly repellent effects of the main constituents of plant essential oils such as 1, 8-cineole, terpineol and α-pinene have also been demonstrated by other researchers (Tapondjou *et al*., 2005; Toloza *et al.* 2006; Nivea *et al*., 2013). Toloza *et al.* (2006) demonstrated strong repellent activity of essential oil from *Eucalyptus cinerea*, *E. viminalis* and *E. saligna*, against permethrin-resistant human head lice. The repellent effect was associated with α-pinene 1, 8-cineole, citronellol, eugenol and camphor. Similarly, *Eucalyptus citriodora* and *Cymbopogon winterianus* oils are repellent to adult *C. maculatus* and repellence was associated with compounds like citronellal, 1, 8-cineole, limonene, geranial, neral, (E)-anethole, and α-pinene (Nivea *et al*., 2013).

Synthetic chemicals are still more frequently used as repellents than essential oils. However, these natural products have the potential to provide efficient and safer repellents to humans and the environment. For many researchers, an effective natural alternative to synthetic repellents will be a highly welcomed innovation. The development will even be a more lucrative idea in stored product pest management where chemical residues and insects in produce may not be tolerated by consumers. In a quest to achieve this noble objective, the repellent potential of *C. lusitanica* and *E. saligna* leaf essential oils were evaluated for instant and residual repellence against adult *T. castaneum, A. obtectus, S. cerealella* and *S. zeamais*.

### 7.2 Materials and Methods

The experimental conditions and methods on rearing of test insects and statistical data analysis are as described in Section 3.1 and 3.2. Likewise, methods dealing with collection and preparations of plant materials hydro-distillation of essential oils, analysis and identification of essential oil constituents are also described in Section 4.2

### 7.2.1 Instant repellence bioassay

The instant repellence test was conducted according to Ogendo *et al.* (2008) and Liang *et al.* (2013) with modifications. The base of a 14-cm diameter plastic Petri dish was lined with aluminum foil, divided into four equal parts and 2.0 g whole/broken wheat (or 4.0 g bean or maize) grain samples placed in each quarter equidistant to the center. Each essential oil was evaluated at five concentrations (0.00, 0.05, 0.10, 0.15 and 0.20% v/w) as an alternate untreated (control)-treated arrangement with four replicates per concentration (Fig. 7.1). Control treatments consisted of a no-choice all untreated and choice bioassays with 5% v/w DEET (N, N-diethyl-*m*-toluamide) and crude soya oil (10.0 $\mu lg^{-1}$). The treated grains were kept for 1 h to allow acetone to evaporate completely. Twenty (20) unsexed adult stages of *A. obtectus, S. zeamais, S. cerealella* and *T. castaneum* were then released at the center of petri-dish and the top secured by its plastic cover. The experimental design and replicates were as described in Section 3.1. The number of insects present in the control ($N_C$) and treated ($N_T$) grains were recorded 1, 3, 5 and 24 h post- exposure. Percent repellence (PR) values were computed according to Asawalam *et al*. (2006)

$$\text{Percent repellence (PR)} = \frac{(N_C - N_T)}{(N_C + N_T)} X100 \qquad (3)$$

### 7.2.2 Residual repellency

Each test essential oil was applied to 20 g wheat (or 40g bean or maize ) grain samples in special self-sealing polythene bags (20 cm x 25 cm; 2L capacity) at five concentrations (0, 0.05, 0.10, 0.15 and 0.20% v/w). The negative control consisted of untreated whereas 5 % v/w DEET (N, N-diethyl-*m*-toluamide) and crude soya oil (10.0 µl/g) were positive controls. The treated grains were transferred to the experimental room for long term storage (120 days). A random sub-sample (2 g wheat and 4 g beans) were then drawn from each experimental unit at 30, 60, 90 and 120 days after treatment (DAT). The experimental design and replicates were as described in section 3.1. Twenty unsexed adults of *A. obtectus, S. zeamais* and *T. castaneum* were then released at the center as described in section 7.2.1. The number of insects present in the control ($N_C$) and treated ($N_T$) grains were recorded after 1, 3, 5 and 24 h exposure. Percent repellents (PR) values were computed according to Asawalam *et al*. (2006) as in section 7.2.1

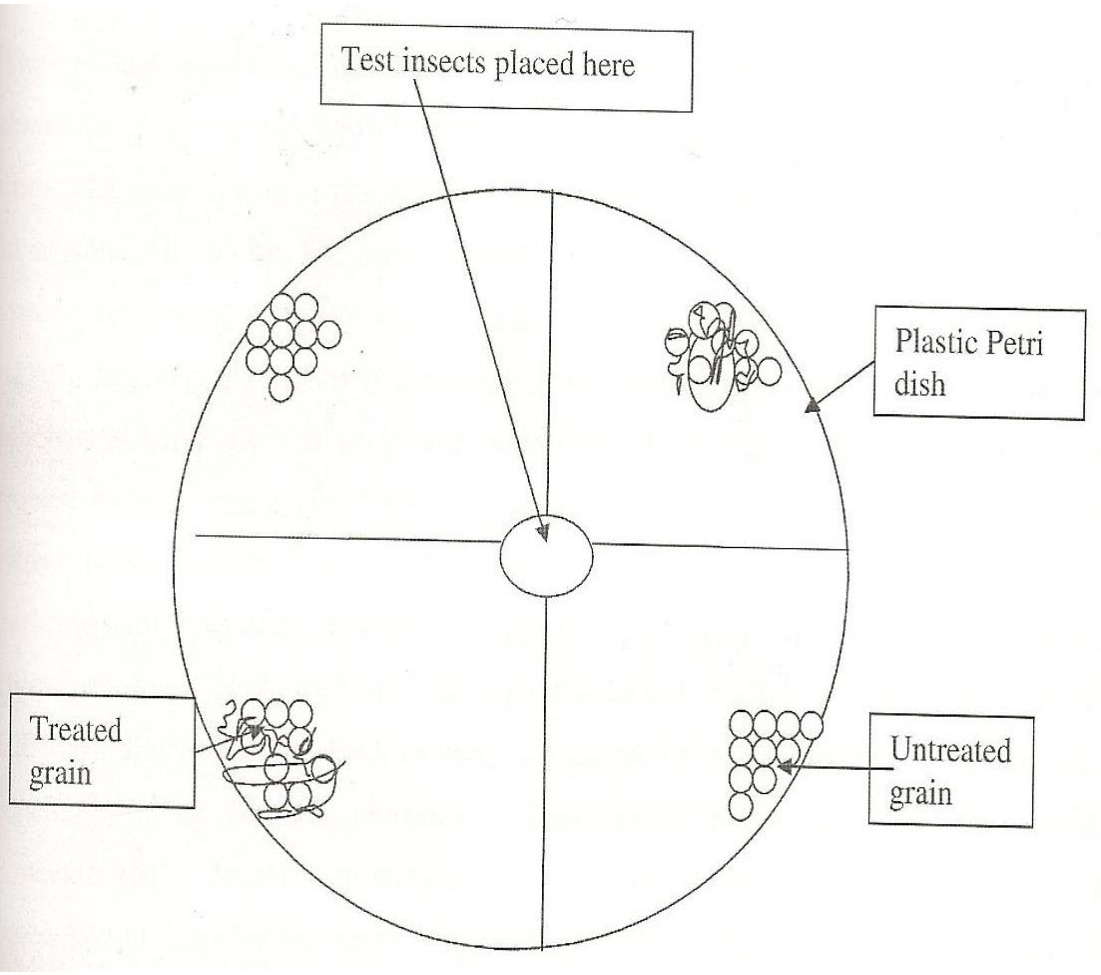



Fig. 7.1: Treated-untreated choice bioassay system

## 7.3 Results

### 7.3.1 Instant repellence

The results of the instant repellence assay for *C. lusitanica* and *E. saligna* leaf essential oils against adult *T. castaneum, A. obtectus, S. cerealella* and *S. zeamais* are presented in Fig.7.2. The plant species, concentration of essential oil applied and time post-treatment significantly influenced the percent repellence (PR) of all the test insects (ANOVA: $F_{(3, 27)} = 4.26 - 63.83$; $P< 0.01- 0.001$) except *A. obtectus* in which all factors were insignificant (ANOVA: $F_{(3, 27)} = 0.431-2.42$; $P> 0.05$).

Data showed that at 0.20% v/w, *C. lusitanica* leaf essential oil was strongly repellent against *T. castaneum* with a PR value of 92.5% but produced low PR values against *A. obtectus* (27.5%) and *S. cerealella* (30.0%) 24 h post-exposure (Fig. 7.2 a & b). At the same concentration, *S. zeamais* showed negative (-5.3%) repellency (attraction) 24 h post-exposure. The PR values for *E. saligna* leaf essential oil, at 0.20% v/w, against *T. castaneum, A. obtectus*, and *S. cerealella* were 9.3, 4.0 and1.8%, respectively 24 h after exposure. *E. saligna* oil was attractant to S. *zeamais* (PR -10%) (Fig. 7.2 a & b). In *T. castaneum* the PR values increased (65-92.5%) with dosage 24 h post-exposure with *C. lusitanica* leaf essential oil. However, both *C. lusitanica* and *E. saligna* leaf essential oils produced decreasing PR values of 12– -4%, 55.5–1.8% and 38.9– -10% against *A. obtectus, S. cerealella* and *S. zeamais*, respectively, 24 post-exposure (Fig. 7.2 a & b). The positive control (DEET-treated) grains produced PR values of 2.5–30.5% in all test insects after 24 h post-exposure, with low repellence observed against *S. zeamais* (30.5%) and *T. castaneum* (27.5%).

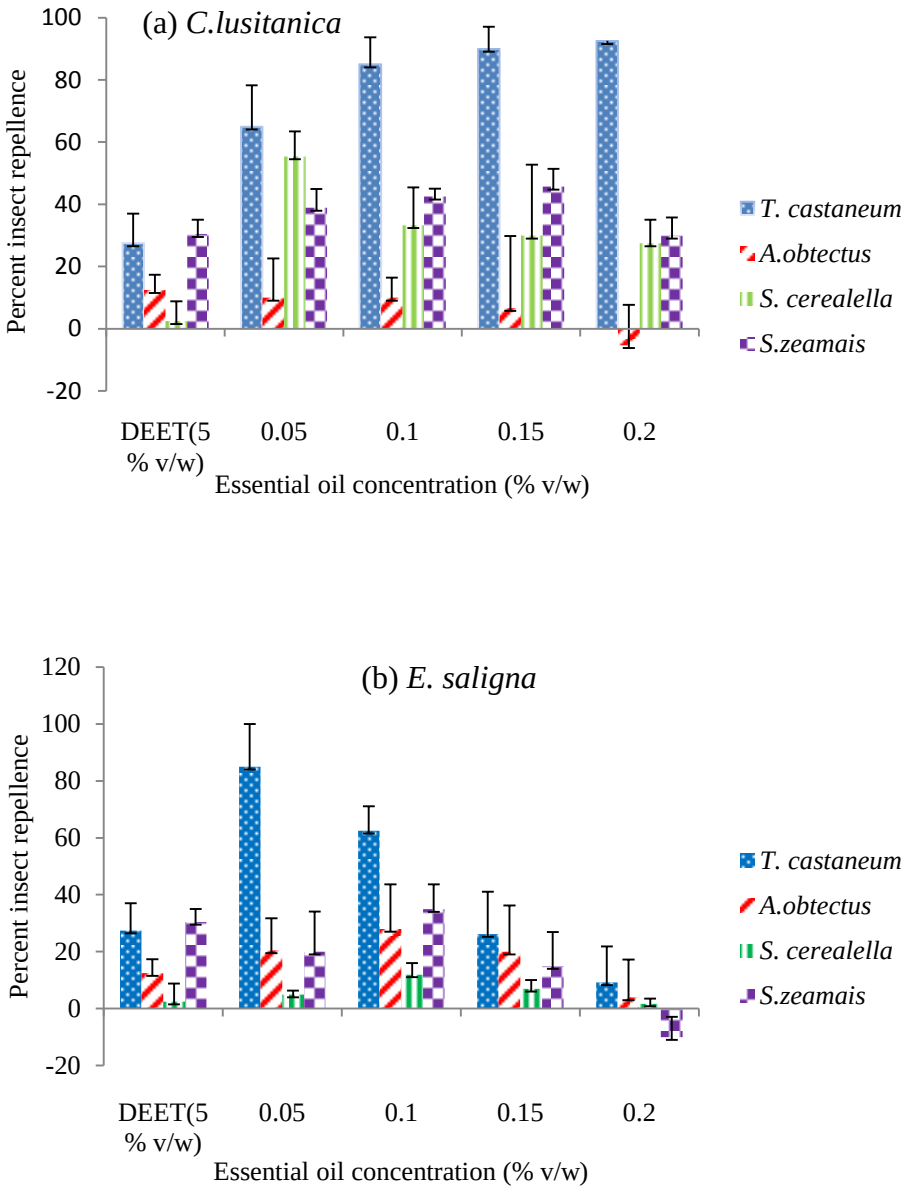



Fig.7.2: End-point percent repellence (Mean ± SE, n=4) of adult *T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais* after 24 h post-exposure to (a) *C. lusitanica* and (b) *E. saligna* leaf essential oils in untreated-treated choice bioassay system(ANOVA output in appendix 8)

Table 7.1: Percent repellence (Mean ± SE, n=4) of adult *T. castaneum, A. obtectus, S. cerealella* and *S. zeamais* 1-24 h post-exposure to *C. lusitanica* leaf essential oils in untreated-treated choice bioassay system

| [a]**Insect species/concentration (% v/w)** | **Exposure Time (h)** | | | |
|---|---|---|---|---|
| | **1** | **3** | **5** | **24** |
| ***T. castaneum*** | | | | |
| [b]DEET (5 % v/w) | -7.3 ± 8.5 | 22.5 ± 6.3 | 25.0 ± 10.4 | 27.5 ± 9.5 |
| 0.05 | 16.4 ± 19.1 | 33.2 ± 4.1 | 25.0 ± 15.0 | 65.0 ± 13.2 |
| 0.1 | 35.0 ± 5.0 | 44.4 ± 6.3 | 55.0 ± 11.9 | 85.0 ± 8.7 |
| 0.15 | 40.0 ± 10.8 | 32.5 ± 7.5 | 47.5 ± 15.5 | 90.0 ± 7.1 |
| 0.2 | 30.0 ± 5.8 | 60.7 ± 14.6 | 33.6 ± 7.3 | 92.5 ± 2.5 |
| ***A. obtectus*** | | | | |
| DEET (5 % v/w) | 39.0 ± 9.9 | 45 ± 2.9 | 34 ± 12.4 | 12.5 ± 4.8 |
| 0.05 | -10.0 ± 10.0 | -3.3 ± 6.4 | -6.7 ± 14.4 | 10.0 ± 12.6 |
| 0.1 | 16.7 ± 10.0 | -10.8 ± 13.4 | -3.3 ± 17.52 | 10.0 ± 6.4 |
| 0.15 | -18.8 ± 7.1 | 6.7 ± 19.6 | 26.7 ± 20.0 | 6.7 ± 23.1 |
| 0.2 | -6.7 ± 5.5 | -3.4 ± 19.2 | 6.7 ± 19.6 | -5.3 ± 12.9 |
| ***S. cerealella*** | | | | |
| DEET (5 % v/w) | 22.5 ± 8.5 | 7.5 ± 8.5 | 12.5 ± 12.1 | 2.5 ± 6.3 |
| 0.05 | 30.8 ± 9.8 | 65.0 ± 20.6 | 51.7 ± 14.7 | 55.5 ± 8.0 |
| 0.1 | 28.2 ± 15.1 | 25.2 ± 18.1 | 30.4 ± 10.5 | 33.3 ± 12.1 |
| 0.15 | 27.6 ± 20.5 | 1.8 ± 19.1 | -9.0 ± 21.3 | 30.0 ± 22.7 |
| 0.2 | -1.6 ± 22.2 | 6.9 ± 10.5 | 6.9 ± 10.5 | 27.5 ± 7.5 |
| ***S. zeamais*** | | | | |
| DEET (5 % v/w) | 12.5 ± 6.5 | 22.5 ± 5.2 | 25.0 ± 1.3 | 30.5 ± 4.5 |
| 0.05 | 15.0 ± 6.9 | 7.0 ± 7.2 | 12.5 ± 1.3 | 38.9 ± 6.0 |
| 0.1 | 32.5 ± 10.5 | 20.5 ± 8.5 | 16.8 ± 5.9 | 42.5 ± 2.5 |
| 0.15 | 30.6 ± 10.6 | 37.5 ± 7.5 | 32.5 ± 8.5 | 45.7 ± 5.7 |
| 0.2 | 15.0 ± 6.5 | 8.7 ± 4.3 | 22.5 ± 7.5 | 30.0 ± 5.8 |

[a]Twenty unsexed adult insects in 4 replicates, were used for each concentration (% v/w)

[b]DEET = N, N-diethyl-*m*-toluamide

ANOVA output in appendix 8

Table 7.2: Percent repellence (Mean ± SE, n=4) of adult *T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais* 1-24 h post-exposure to *E. saligna* leaf essential oils in untreated-treated choice bioassay system

| [a]**Insect species/conc.(% v/w)** | **Exposure Time (h)** | | | |
|---|---|---|---|---|
| | **1** | **3** | **5** | **24** |
| ***T. castaneum*** | | | | |
| [b]DEET (5 % v/w) | -7.3 ± 8.5 | 22.5 ± 6.3 | 25 ± 10.4 | 27.5 ± 9.5 |
| 0.05 | 37.5 ± 11.1 | 15.0 ± 24.7 | 30.3 ± 12.3 | 85.0 ± 15.0 |
| 0.1 | 15.5 ± 9.4 | 25.0 ± 2.9 | 48.0 ± 6.6 | 62.5 ± 8.5 |
| 0.15 | -7.8 ± 19.9 | 7.5 ± 8.5 | 2.5 ± 16.0 | 26.2 ± 14.9 |
| 0.2 | -43.0 ± 6.7 | -30.5 ± 14.2 | -29.8 ± 11.2 | 9.3 ± 12.6 |
| ***A. obtectus*** | | | | |
| DEET (5 % v/w) | 39 ± 9.9 | 45 ± 2.9 | 34 ± 12.4 | 12.5 ± 4.8 |
| 0.05 | 8.7 ± 22.7 | 3.6 ± 22.4 | -7.6 ± 7.4 | 20.5 ± 11.2 |
| 0.1 | -15.0 ± 13.2 | -5.3 ± 18.4 | -4.3 ± 20.8 | 28.0 ± 15.7 |
| 0.15 | -15.0 ± 10.5 | -20.9 ± 22.8 | -20.4 ± 16.3 | 20.0 ± 16.2 |
| 0.2 | -27.5 ± 5.8 | 9.0 ± 4.3 | -2.8 ± 20.3 | 4.0 ± 13.3 |
| ***S. cerealella*** | | | | |
| DEET (5 % v/w) | 22.5 ± 8.5 | 7.5 ± 8.5 | 12.5 ± 12.1 | 2.5 ± 6.3 |
| 0.05 | 3.3 ± 4.3 | 5.0 ± 15.8 | 17.5 ± 11.1 | 5.0 ± 1.3 |
| 0.1 | 30.0 ± 14.1 | 8.8 ± 4.2 | 12.5 ± 14.9 | 12.0 ± 4.0 |
| 0.15 | 25.0 ± 13.2 | 2.5 ± 8.5 | 17.5 ± 4.8 | 7.0 ± 3.0 |
| 0.2 | -7.5 ± 10.3 | -12.5 ± 8.0 | -7.5 ± 4.8 | 1.8 ± 1.8 |
| ***S. zeamais*** | | | | |
| DEET (5 % v/w) | 12.5 ± 6.5 | 22.5 ± 5.2 | 25 ± 1.3 | 30.5 ± 4.5 |
| 0.05 | 3.3 ± 4.3 | 5.0 ± 15.8 | 17.5 ± 11.1 | 20.0 ± 14.1 |
| 0.1 | 30.0 ± 14.1 | 8.8 ± 4.2 | 12.5 ± 14.9 | 35.0 ± 8.7 |
| 0.15 | 25.0 ± 13.2 | 2.5 ± 8.5 | 17.5 ± 4.8 | 15.0 ± 11.9 |
| 0.2 | -7.5 ± 10.3 | -12.5 ± 8.0 | -7.5 ± 4.8 | -10.0 ± 7.1 |

[a]Twenty unsexed adult insects in 4 replicates, were used for each concentration (% v/w)

[b]DEET = N, N-diethyl-*m*-toluamide

(ANOVA output in appendix 8)

### 7.3.2 Residual repellence

#### 7.3.2.1 Residual repellence of *C. lusitanica* essential oils

Results of residual repellence of *C. lusitanica* leaf essential oils against *S. zeamais, T. castaneum* and *A. obtectus* after 30-120 days of grain storage are presented in Fig 7.3. The *C. lusitanica* leaf essential oils produced a dose-, grain storage duration- and exposure time-dependent percent residual repellence against adult *T. castaneum* (ANOVA: $F_{(3, 3)} = 3.4\text{-}6.6$; $P< 0.01 – 0.001$, , *A. obtectus* (ANOVA: $F_{(3, 3)} = 5.6\text{-}9.2$; $P< 0.001$,) and *S. zeamais* (ANOVA: $F_{(3, 9)} = 3.51\text{-}20.9$; $P< 0.001$). Data also showed that, at the highest concentration of 0.20% v/w and 30 days grain storage duration, *C. lusitanica* leaf essential oil was moderately repellent to *S. zeamais* (49.3%) but produced low PR values against *T. castaneum* (13.2%) and A. *obtectus* (32.2%) 12 h post-introduction of test insects. At the same concentration and 120 days grain storage duration, the oil was moderately repellent with PR values of 37.9, 47.6 and 51.1% against adult *A. obtectus. S. zeamais* and *T. castaneum*, respectively 12 h post-introduction of test insects.

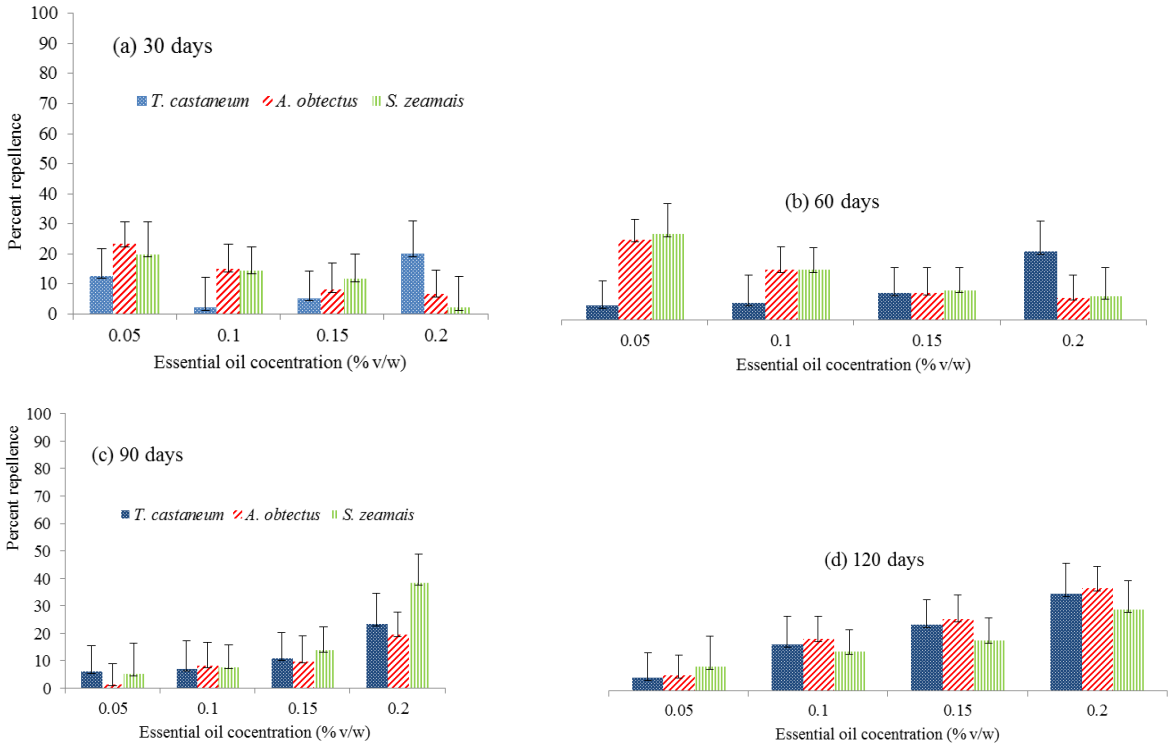



Fig.7.3: Percent repellence (Mean ± SE, n=4) of *C. lusitanica* essential oils against adult *T. castaneum*, *A. obtectus* and *S. zeamais* 12 h post-exposure of test insects and in (a) 30 days (b) 60 days (c) 90 days and (d)120 days grain storage duration(ANOVA output in appendix 9).

### 7.3.2.2 Residual repellence of *E. saligna* leaf essential oils

Data of residual repellence of *E. saligna* leaf essential oils against *S. zeamais, T. castaneum* and *A. obtectus* after 30-120 days grain storage duration are presented in Fig.7.4. The *E. saligna* leaf essential oils produced dose-, grain storage duration- and exposure time-dependent residual PR against adult *T. castaneum* (ANOVA: $F_{(3, 9)} = 2.2\text{-}9.1$; $P< 0.05\text{-}0.001$ and *S. zeamais* (ANOVA: $F_{(3, 9)} = 1.7\text{-}13.4$; $P< 0.05\text{-}0.001$) except *A. obtectus* in which all factors were insignificant (ANOVA: $F_{(3, 9)} = 0.4\text{-}3.7$; $P> 0.05$). The PR values for *E. saligna* essential oils, at 0.20 % v/w and 30 days grain storage duration, against adult *A. obtectus, S. zeamais* and *T. castaneum* were 17.8%, 22.9% and 33.6%, respectively, 12 h post-introduction of test insects. Similarly, at the same concentration and 120 days grain storage duration; oil was moderately repellent with a PR value of 52.4% in *T. castaneum* but weakly repellent to *A. obtectus* (34.0%) and *S. zeamais* (36.6%), 12 h post-introduction of test insects.

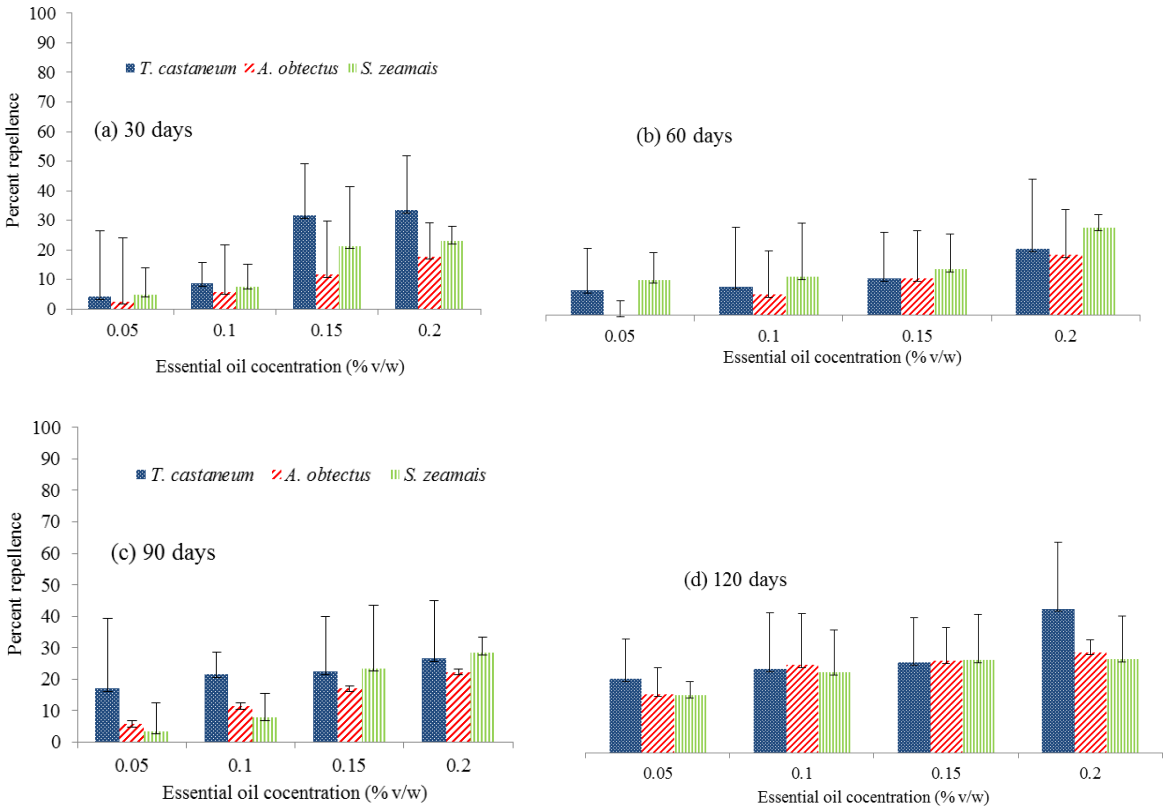



Fig.7.4: Percent repellence (Mean ± SE, n=4) of *E. saligna* essential oils against adult *T. castaneum*, *A. obtectus* and *S. zeamais* 12 h post-exposure of test insects and in (a) 30 days (b) 60 days (c) 90 days and (d)120 days grain storage duration(ANOVA output in appendix 10).

## 7.4 Discussion

The results of instant and residual repellency assays of leaf essential oils of *C. lusitanica* and *E. saligna* against test insects showed variable responses. However, repellence was influenced by insect and plant species, concentration of oil exposure time and storage duration. Results on instant repellence have shown clearly that *C. lusitanica* essential oil was a strong repellent against *T. castaneum* at a concentration of 0.20 % v/w after 24 h of exposure and moderately repellent against *S. zeamais*. *E. saligna* oil was a poor repellent in all test insects even at higher concentrations and longer exposure periods.

These results are in agreement with previous local studies in which instant repellency depended on intra-species, intra-plant variations, concentration, insect species (Ogendo, 2008). Essential oils obtained from *L. camara*, *O. americanum*, and *T. vogelii* were effective repellents against *S. oryzae*, *T. castaneum*, *C. chinensis* and *R. dominica* with PR values in the range of 60-83% (Ogendo *et al*, 2008b). Chebet *et al*. (2013) demonstrated that grains treated with crude powders of *T. vogelii* and *A. indica* were equally the most repellent (PR values: 88–90%) against adult *P. truncatus* followed by *Lantana camara* (PR 73%). Tapondjou *et al*. (2005) reported essential oils and cymol obtained from *Eucalyptus saligna* and *C. sempervirens*, to have repellent and toxic effects on *S. zeamais* and *T. castaneum*. The observed variable repellent activity could partly be attributed to the presence of volatile constituents such as monoterpenes and sesquiterpenes which are well-known repellents of phytophagous (biting) insects by acting in the vapour form on the olfactory receptors (Lee *et al*., 2003;Wang *et al*., 2006). The highly repellent effects of the main constituents of essential oils such as 1, 8-cineole, terpineol and a-pinene have been demonstrated (Tapondjou *et al*., 2005). Despite not being tested directly the repellent activity of the essential oils in current study may be attributed to major constituents of *C. lustanica* oil such as α-pinene, δ-3-carene, terpien-4-ol, phellandrene, *cis*-cadina-1(6), 4-diene, α- cedrene and *trans*-muurola-4(14), 5-diene. Similarly, repellence of *E. saligna* oil could be linked to its major compounds like borneol, α- terpineol, α-pinene , *p*-cymene , α- guaiene, *iso*-leptospermone and spathulenol.

It is also evident from results of this residual repellence study that *C. lusitanica* and *E. saligna* essential oils are weak residual repellents against test insects (PR 27.5-30%) 24 h post-exposure.

The results indicate also that repellence decreased with dosage and even negative repellence (attraction) observed. It was also observed that in residual repellence assay percent repellence increased with exposure time in all test insects. The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. Due to their high volatility they may be lost after long exposure periods (Regnault-Roger, 2012). Similar results trend were also observed by Wambua *et al.* (2011) who reported a dose- and exposure time-dependent negative repellence (attraction) of *H. armigera* larvae to chickpea leaves treated with aqueous extracts of *T. vogelii*. Ogendo *et al*. (2003) reported that maize grains admixed with Actellic Super$^{TM}$ 2% dust registered negative PR values against *S. zeamais* due to the arrestment of test insect by the chemical. In similar studies, Ogendo *et al* (2008b) reported eugenol produced PR values that decreased with dosage of *C. chinensis* on treated grains. The major cause of the negative PR values was possibly due to the high contact toxicity of eugenol (Ogendo *et al*., 2008b) against *C. chinensis*.

The positive results of the repellence of *T. castaneum* by *C. lusitanica* and *E. saligna* essential oils is an exciting scientific development since these pest have been shown to the tolerate toxic effects of essential oils. Diversification of approaches for control of insect pests can achieve better results. This could be done by carrying out several treatment diversifying the biochemical targets in the insect and using genetic engineering, physical and chemical methods and entomophagous control. The combination of all these methods used simultaneously or alternately, would certainly decrease the undesirable and secondary effects of pests and pathogens and also reduce the amounts of insecticide employed.

However, *C. lusitanica* and *E. saligna* essential oils provide nothing significant as far as an effective repellent against *A. obtectus*, *S. zeamais* and *S. cerealella* is concerned. However, negative repellence could scientifically be exciting especially in the push-pull strategy in integrated pest management where a protected source (crop) is unsuitable to pest (Push) while luring towards an attractive source (Pull) from where the pests are subsequently removed or killed avoiding residues in crop (Cook *et al*., 2007).

The current study has identified some possible alternative botanical insecticides and repellents to replace synthetics ones currently in use. Moreover, provided with a proper formulation and dosage, and sufficient regulatory frame work, the plant essential oils may be exploited for use against insect infestation at the small scale farmer's level since they may be more effective and less cumbersome than application of dangerous synthetics. If the problem of cost-effective commercial production can be solved, some of the compounds tested could find a place in IPM strategies, especially where the emphasis is on environmental and food safety and on replacing the more hazardous synthetic repellents and insecticides.

# CHAPTER EIGHT

# REPRODUCTION INHIBITION RATES OF *Cupressus lusitanica* AND *Eucalyptus saligna* ESSENTIAL OILS AGAINST *Tribolium castaneum, Acanthoscelides obtectus* AND *Sitophilus zeamais*


## Abstract

A laboratory study was conducted to evaluate reproductive inhibition rate of *C. lusitanica* and *E. saligna* essential oils in reducing progeny of *T. castaneum, A. obtectus and S. zeamais*. Hydro-distilled essential oils of test plants were evaluated at five concentrations of 0, 0.05, 0.10, 0.15 and 0.20% v/w. Twenty (20) unsexed adult insects were allowed to lay eggs for 5 days on beans and wheat grains and adults removed. Numbers of emerging adults were recorded 5, 10, 15 and 20 days post- emergence of the first adults and inhibition rates computed. In total 6, 15.4 and 23 adult *T. castaneum* emerged from *C. lusitanica* oil treated grains, *E. saligna* oil treated grains and untreated control, respectively. In *S. zeamais*, 54, 57 and 131 adults emerged from *C. lusitanica*, *E. saligna* oil treated grains and untreated control respectively. In both oils no progeny emerged in *A. obtectus* as compared to 22 in untreated control. Percent progeny reduction in *C. lusitanica* oil treated grains was 50, 52 and 100% in *T. castaneum, S. zeamais*, and *A. obtectus*, respectively. *E. saligna* oils caused 58, 79 and 100% reductions in first generation progeny of *S. zeamais, T. castaneum* and *A. obtectus*, respectively. The test essential oils could have reduced fecundity, decreased egg hatchability, caused larval mortality and adversely influenced offspring emergence in test insects. Results point to *C. lusitanica* and *E. saligna* essential oils as potential reproduction inhibitors of stored product insect pests and candidate botanical insecticides for possible inclusion in insect pest management options.



**Key words:** essential oil, inhibition rate, monoterpenoids, progeny reduction


## 8.1 Introduction

Insect damage to stored cereals and legumes is the concern of many farmers and food security experts in many tropical countries (Ogendo *et al*., 2011). Storage pests such as maize weevil (*S. zeamais*), Rice weevil (*S. oryzae*), Angoumis grain rain moth (*S. cerealella*), Lesser grain borer (*R. dominica*), Red rust flour beetle (*T. castaneum*), bean bruchid (*A. obtectus*), cowpea beetle (*C. chinensis*) and others cause quantitative and qualitative loss to grains (Nukenine, 2010;

Kumar *et al.* 2011). Quantitative loss due to grain weight loss and qualitative due to loss of nutritional and aesthetic value has led to food insecurity and economic loss globally. The problem related to agricultural pests is more pronounced in the tropical countries, due to agro-climatic conditions and lack of adequate storage facilities (Kumar *et al.*, 2011). In addition, the widespread and intensive use of synthetic insecticides for the control of stored grain insects has led to serious set-backs including insecticide resistance, poisoning of handlers, rising cost of production, lethal effects on non-target organisms and environmental pollution. In the present scenario, protection of stored grains and agricultural products from insect infestation using less toxic, low cost and effective methods is, an urgent goal.

In this direction, many plant essential oils and constituents have been evaluated for their toxic, anti-feedant, repellent and reproductive inhibition properties against different stored grain pests (Kumar *et al*., 2011; Ogendo *et al*., 2012). Essential oils and constituents are active against both adults and larvae and frequently act to inhibit reproduction (Kumar *et al*., 2011; Regnault-Roger *et al*., 2012). The ability of essential oils and monoterpenoids to reduce fecundity and progeny in different stored insects has already been reported (Ogendo 2008; Alzogaray *et al*., 2011; Gomah *et al.*, 2015). Papachristos and Stamopoulos (2002) reported essential oils of 13 plants belonging to Umbelliferae, Rutaceae, Myrtaceae, Cupressaceae,Lauraceae, Labiatae and Anacardiaceae families to have repellent action, reduced fecundity, decreased egg hatchability, increase neonate larval mortality and adversely influence offspring emergence in *A. obtectus*. In similar studies, Asawalam and Hassanali (2006) found *Vernonia amygdalina* essential oil to have significantly reduced the number of progeny produced by *S. zeamais,* and induced a high repellent action against the weevil. In local studies, essential oils extracted from aerial parts of *T. vogelli, L. camara* and *O. americanum* were shown by Ogendo (2008) to have reproductive inhibitory effects of *S. oryzae, C. chinensis* and *R. dominica.*

In the public health sector, Sedaghat *et al.* (2011) reported that the leaf essential oil of *Cupressus arizonica* has larvicidal activity against fourth instar larvae of laboratory-reared *An. stephensi*. The essential oils of six plant species *Mentha piperita, Mentha citrate, Eucalyptus globulus, Cymbopogon citratus, Vetiver zizanoides* and *Curcuma longa* were reported to have repellent, larvicidal and pupicidal activities against the housefly, *Musca domestica* L. (Kumar *et al*, 2011). Similarly**,** Priyanka and Ayesha (2013) reported mixtures of plant oils (cedarwood +

eucalyptus oil, cedarwood + peppermint oil and (cedarwood+ camphor) to exhibit high reproductive inhibition in 4th instar larvae of *Corcyra cephalonica.*

The reproductive inhibition effects of constituents of essential oils such as 1, 8-cineole, *p*-cymene, and γ-terpinene and α-pinene have also been earlier demonstrated (Sedaghat *et al*., 2011; Alzogaray *et al.*, 2011; Gomah *et al.*, 2015). *Cupressus arizonica* essential oil had larvicidal activity against fourth instar larvae of laboratory-reared *An. stephensi* with $LC_{50}$ and $LC_{90}$ values of 79.30 ppm and 238.89 ppm, respectively. Essential oil contained limonene (14.44%), umbellulone (13.25%) and α-pinene (11%) were determined as the main constituents (Sedaghat *et al*., 2011). In the same way, essential oils from 11 species of the genus *Eucalyptus* were found to have larvicidal effects on first instar of *Blattella germanica* L. with knockdown time 50% ($KT_{50}$) of 38.8-178.3 minutes in monoterpenes α-pinene, 1,8-cineole, *p*-cymene, and γ-terpinene (Alzogaray *et al*., 2011).

The reported reproductive inhibition effect of essential oils and constituents as sources of potentially efficient insecticides is an exciting finding. Therefore, the prospects of application of these natural growth regulators in insect pest control options in stored products may be of interest for further research. In the current study, it was considered of scientific interest to evaluate reproductive inhibition rates of *C. lusitanica* and *E. saligna* essential oils in *T. castaneum, A. obtectus* and *S. zeamais*.

## 8.2 Materials and methods

The experimental conditions and methods on rearing of test insects and statistical data analysis are as described in section 3.1 and 3.2. Likewise, methods dealing with collection and preparations of plant materials hydro-distillation of essential oils, analysis and identification of essential oil constituents are also described in section 4.2.

### 8.2.1 Reproduction inhibition test

A Laboratory bioassay for reproductive inhibition effects (progeny studies) was conducted according to the method of Ogendo (2008) and Kumar *et al*. (2011) with modifications. Whatman filter paper disks (90 mm in diameter) were soaked in essential oils at concentrations of 0, 0.05, 0.10, 0.15 and 0.20% v/w and air–dried for an hour to allow solvent to evaporate. The

control filter papers contained untreated, crude soya oil and actellic super $^{TM}$ 5EC. The treated and control filter paper discs were placed singly at the bottom of Petri dishes (90 mm diameter ) and 20 g of wheat or bean grain placed on filter papers. Twenty unsexed adult *A. obtectus, S. zeamais* and *T. castaneum* were obtained by sieving grain using an entomological sieve, placed in each Petri dishes which were then

covered for the next 5 days to allow tests insects to lay eggs. The experimental design and replicates were as described in Section 3.1. The adults were then removed and the number of emerging adult recorded 5, 10, 15 and 20 days post- emergences of the first adults. The first emergence of *T. castaneum* was on 25$^{th}$ day, *A. obtectus* was 28$^{th}$ and *S. zeamais* 35$^{th}$ day post-set up of experiment. The percentage reduction in adult emergence or inhibition rate was (IR) computed as follows (Kumar *et al*., 2011):

$$\text{Percent inhibition rates (IR)} = \frac{C_n - T_n}{C_n} X100 \qquad (4)$$

Where $C_n$ = Number of insects in control dish and $T_n$ = Number of insects in Treated dish

**8.3 Results**

The results of reproductive inhibition of *C. lusitanica* and *E. saligna* leaf essential oils against four test insects revealed significant plant-, essential oil concentration- and time- dependent progeny reduction (ANOVA: $F_{(1, 9)} = 2.35 - 34.51$; $P< 0.05- 0.001$). Table 8.1 shows the number of adult insects that emerged 20 days post-emergence of first generation progeny in negative and positive control treatments and essential oils of test plants at a dose of 0.20% v/w. The number of progeny emerged from soya oil treated grains were on average 1, 2.3 and 4.5 in *A. obtectus*, *S. zeamais* and *T. castaneum*, respectively. In actellic super$^{TM}$ treatment, no *A. obtectus* and *S. zeamais* progeny emerged but in *T. castaneum* 2.8 progeny emerged. In the essential oil treatments (0.20 % v/w), 6 and 15.4 *T. castaneum* adults emerged respectively from *C. lusitanica* and *E. saligna* oil treated grains as compared to 23 adults in the untreated control. Similarly, 54 and 57 *S. zeamais* adults emerged respectively from *C. lusitanica* and *E. saligna* oil treated grains as compared to 131 adults in the untreated control. In *A. obtectus* where no progeny emerged after treatment with both oils as compared to 22 in untreated control.

The results of reproductive inhibition test of *C. lusitanica* essential oils against, *T. castaneum, A. obtectus* and *S. zeamais* after adult emergence duration of 5-20 days are presented in Table 8.2. At the highest concentration of 0.20 % v/w and 20 days post-emergence of first progeny *C lusitanica* leaf essential oils caused 50. 52 and 100 % reductions in progeny of *T. castaneum, S. zeamais,* and *A. obtectus*, respectively. There was *C. lusitanica* essential oil concentration-dependent percent reduction in the first generation progeny of 34. 4-50.0, 39.9-59.9 and 99.4-100% in *T. castaneum, S. zeamais,* and *A. obtectus*, respectively.

The results also show that, at 0.20% v/w and 20 days post-emergence of first progeny, *E. saligna* oils caused 58, 79 and 100% reductions in first generation progeny of *S. zeamais, T. castaneum* and *A. obtectus*, respectively. Similarly, there was an increase in percent progeny reduction with concentration of *E. saligna* essential oil of 22 -57.9, 54.3-79.4, and 80-100% in *T. castaneum, S. zeamais,* and *A. obtectus*, respectively. In comparison, *E. saligna* oil was a better reproduction inhibitor of *T. castaneum* than *C. lusitanica.* However, the two oils were equally effective in first generation progeny reduction; 58-59% in *S. zeamais* and 100% in *A. obtectus*.

Table 8.1: Number (Mean ± SE, n = 4) of adult insects (*T. castaneum*, *A. obtectus* and *S. zeamais*) that emerged within 5-20 days emergence time (days) in control treatments and 0.20 % v/w of *C. lusitanica* and *E. saligna* essential oils

| [a]Insect/concentration | Emergence Time (Days) 5 | 10 | 15 | 20 |
|---|---|---|---|---|
| ***T. castaneum*** | | | | |
| 0 % v/w (untreated control) | 0.25 ± 0.3 | 0.5 ± 0.3 | 1.3 ± 0.5 | 23.4 ± 2.5 |
| Actellic super™ (0.056 % w/w) | 0.5 ± 0.3 | 1.3 ± 0.5 | 2.0 ± 0.7 | 2.8 ± 0.9 |
| Soya oil(10 % v/w) | 0.25 ± 0.3 | 0.25 ± 0.3 | 0.5 ± 0.3 | 4.5 ± 0.5 |
| *C. lusitanica* oil (0.2 % v/w) | 0 | 1 ± 0.7 | 7.3 ± 1.2 | 15.5 ± 1.9 |
| *E. saligna oil* (0.2 % v/w) | 0 | 0.8 ± 0.5 | 1.5 ± 0.5 | 6.0 ± 0.7 |
| ***A. obtectus*** | | | | |
| 0 % v/w (untreated control) | 0.25 ± 0.3 | 8.8 ± 2.6 | 14.5 ± 4.6 | 22.3 ± 5.9 |
| Actellic super™ (0.056 % w/w) | 0 | 0 | 0 | 0 |
| Soya oil(10 % v/w) | 0.25 ± 0.3 | 0.5 ± 0.3 | 0.8 ± 0.5 | 1 ± 0.5 |
| *C. lusitanica* oil(0.2 % v/w) | 0 | 0 | 0 | 0 |
| *E. saligna* (0.2 % v/w) | 0 | 0 | 0 | 0 |
| ***S. zeamais*** | | | | |
| 0 % v/w (untreated control) | 19.3 ± 0.5 | 48.8 ± 2.3 | 91 ± 0.5 | 131 ± 2.4 |
| Actellic super™ (0.056 % w/w) | 0 | 0 | 0 | 0 |
| Soya oil(10 % v/w) | 0.25 ± 0.3 | 0.5 ± 0.3 | 1.5 ± 0.6 | 2.3 ± 1.0 |
| *C. lusitanica* oil (0.2 % v/w) | 11.3 ± 1.3 | 23 ± 2.8 | 41.5 ± 2.5 | 54.3 ± 5.3 |
| *E. saligna* oil (0.2 % v/w) | 11.5 ± 1.8 | 23 ± 1.2 | 39.3 ± 1.4 | 56.8 ± 1.5 |

[a]Twenty unsexed adult insects in 4 replicates, were used for each concentration (ANOVA output in appendix 11)

Table 8.2 Percent reduction (Mean ± SE, n = 4) of progeny of *T. castaneum, A. obtectus* and *S. zeamais* within 5-20 days emergence time (days) after grains were treated with concentrations of 0.05-0.20 % v/w of (a) *C. lusitanica* and (b) *E. saligna* essential oils

(a) *C. lusitanica* oil

| [a]Insect/conc. (% v/w) | Emergence Time (Days) 5 | 10 | 15 | 20 |
|---|---|---|---|---|
| ***T. castaneum*** | | | | |
| 0.05 | 100.0 | 66.7 ± 16.0 | 61.8 ± 23.2 | 35.4 ± 12.5 |
| 0.1 | 100.0 | 75.0 ± 8.3 | 61.8 ± 6.9 | 38.8 ± 3.6 |
| 0.15 | 100.0 | 83.3 ± 16.7 | 67.1 ± 7.5 | 46.6 ± 7.8 |
| 0.2 | 100.0 | 91.7 ± 23.6 | 85.5 ± 6.2 | 50 ± 6.7 |
| ***A. obtectus*** | | | | |
| 0.05 | 96.0 ± 3.9 | 98.5 ± 1.6 | 99.1 ± 0.9 | 99.4 ± 0.6 |
| 0.1 | 100.0 | 100.0 | 100.0 | 100.0 |
| 0.15 | 100.0 | 100.0 | 100.0 | 100.0 |
| 0.2 | 100.0 | 100.0 | 100.0 | 100.0 |
| ***S. zeamais*** | | | | |
| 0.05 | 18.8 ± 3.8 | 33.6 ± 2.2 | 39.8 ± 1.9 | 39.9 ± 3.2 |
| 0.1 | 27.5 ± 7.2 | 40 ± 7.0 | 48.1 ± 3.2 | 50.2 ± 3.7 |
| 0.15 | 26.8 ± 10.3 | 44.5 ± 5.1 | 49.5 ± 2.8 | 55.8 ± 4.1 |
| 0.2 | 42.5 ± 6.6 | 57.3 ± 5.1 | 58.9 ± 3.2 | 59.8 ± 4.0 |

[a]Twenty unsexed adult insects in 4 replicates, were used for each concentration
ANOVA output in appendix 11

(b) *E. saligna* oil

| [a]Insect/conc. (% v/w) | Emergence Time (Days) 5 | 10 | 15 | 20 |
|---|---|---|---|---|
| ***T. castaneum*** | | | | |
| 0.05 | 87.5 ± 12.5 | 62.5 ± 23.9 | 45 ± 5.0 | 54.3 ± 6.1 |
| 0.1 | 100.0 | 75 ± 25 | 70 ± 12.6 | 62.1 ± 4.5 |
| 0.15 | 100.0 | 100.0 | 75 ± 22.2 | 62.9 ± 6.7 |
| 0.2 | 100.0 | 100.0 | 95 ± 17.3 | 79.4 ± 2.4 |
| ***A. obtectus*** | | | | |
| 0.05 | 96.2 ± 3.9 | 95.4 ± 1.6 | 86.6 ± 1.7 | 80 ± 1.0 |
| 0.1 | 100.0 | 95.4 ± 4.7 | 94.6 ± 4.3 | 91.8 ± 3.3 |
| 0.15 | 100.0 | 100.0 | 100.0 | 99.4 ± 0.6 |
| 0.2 | 100.0 | 100.0 | 100.0 | 100.0 |
| ***S. zeamais*** | | | | |
| 0.0®5 | 16.3 ± 9.0 | 25.5 ± 3.9 | 33.5 ± 2.9 | 22.2 ± 7.0 |
| 0.1 | 25 ± 5.4 | 350 ± 4.7 | 41.8 ± 2.9 | 40.8 ± 3.6 |
| 0.15 | 32.5 ± 8.8 | 49.1 ± 3.7 | 53.5 ± 1.2 | 51.3 ± 1.3 |
| 0.2 | 42.5 ± 9.2 | 57.7 ± 2.2 | 61.15 ± 1.4 | 57.9 ± 1.1 |

[a]Twenty unsexed adult insects in 4 replicates, were used for each concentration
ANOVA output in appendix 11

## 8.4 Discussion

The results of the reproduction inhibition bioassays, with *C. lusitanica* and *E. saligna* essential oils against test insects varied with essential oil concentration, insect species and corresponding factor interactions. The *C lusitanica* and *E. saligna* oils at concentrations of 0.20% v/w reduced (50-100 %) drastically the number of emerging progeny in *S. zeamais* and *T. castaneum* and even there were no progeny emerging in *A. obtectus*. It is clear that *C. lusitanica* and *E. saligna* oils are efficient reproductive inhibitors with a progeny reduction of more than 50% in all test insects. In addition *E. saligna* oil was a better reproductive inhibitor of *T. castaneum* than *C. lusitanica* oil.

The findings of the current study concur with several previous investigations in which various plant essential oils which caused significant reproduction inhibition effects against stored product insect pests (Papachristos and Stamopoulos, 2004; Asawalam *et al*., 2006; Ogendo, 2008; Gomah *et al.,* 2015). Papachristos and Stamopoulos (2002) reported that essential oils obtained from 13 different plant species had repellent action, reduced fecundity, decreased egg hatchability, increased neonate larval mortality and adversely influenced offspring emergence in *A. obtectus*. In *S. zeamais,* plant powders from *Piper guineense* and *Capsicum frutescens* reduced adult emergence, grain damage and weight loss and essential oil of *Vernonia amygdalina* at a dose of 750 mg (0.3%) produced no progeny (Asawalam and Hassanali, 2006; Asawalam *et al*., 2007). In local studies, Ogendo (2008) reported essential oils extracted from aerial parts of *T. vogelli, L. camara* and *O. americanum* caused 42-68, 35-60, and 28-61% reductions in progeny, respectively of *S. oryzae*, *C. chinensis* and *R. dominica.* In a more recent study, essential oils obtained from *Ageratum conyzoides*, *Achillea fragrantissima* and *Tagetes minuta* were proven to have ovicidal and adulticidal effects against *C. maculatus* with $LC_{50's}$ of 71.6 - 161.9 $\mu lL^{-1}$ air and 19.2–77.8 $\mu lL^{-1}$ air against eggs and adults, respectively following a 24-h fumigation and a 48-h post exposure period (Gomah *et al.,* 2015).

There are numerous reports on reproductive inhibitory effects of constituents of essential oils such as 1, 8-cineole, *p*-cymene, and γ-terpinene and α-pinene (Sedaghat *et al*., 2011; Alzogaray *et al*., 2011; Gomah *et al.,* 2015). Despite not being tested directly, the reproduction inhibitory activity of the essential oils in the current study may be attributed to major constituents of *C.*

*lustanica* oil such as α-pinene, δ-3-carene, terpien-4-ol, phellandrene, *cis*-cadina-1(6), 4-diene (1.6 %) α- cedrene (2.6%) and *trans*-muurola-4(14), 5-diene. Similarly, progeny reduction could also be associated with *E. saligna* essential oil constituents like borneol, α- terpineol, α-pinene , *p*-cymene , α- guaiene, *iso*-leptospermone and spathulenol. Sedaghat *et al*. (2011) reported *Cupressus arizonica* essential oil containing mainly limonene, umbellulone and α-pinene to had larvicidal activity against fourth instar larvae of laboratory-reared *An. stephensi* with $LC_{50}$ and $LC_{90}$ values of 79.30 ppm and 238.89 ppm, respectively.

These results, and those reported earlier, indicate that the insecticidal activity of the essential oils varies depending on the stage of the insect development, the species and the plant origin of the essential oil (Negahban *et al*., 2006). The growth regulator effect of essential oils can be understood as malfunctioning of insect metamorphosis which may be either completely inhibition or prevent to occur at the right time. Some extracts , termed insect growth regulators (IGRs), can have pronounced effect on developmental period , growth, adult emergence, fecundity , fertility and egg hatching resulting in effective control (Kumar *et al*., 2011). Other phytochemicals have shown growth inhibiting effects such as prolongation of instar and pupae durations, inhibition of larval and pupal moulting, morphological abnormalities and mortality especially during moulting (Kumar *et al*., 2011; Regnault-Roger, 2012).

It is clear from results of the present investigation that essential oils and constituents of *C. lusitanica* and *E. saligna* are promising reproductive inhibitors that may be used in the control of coleopteran and lepidopteran pests of stored products. If the cost-effective commercial production and standardization issues are addressed, the essential oils obtained from these plants can possibly play a role in integrated pest management strategies in smallholder agriculture.

# CHAPTER NINE
# GENERAL DISCUSSION

The sustained control of insect pests using synthetic insecticides has produced adverse secondary effects including acute and chronic poisoning of applicators, farmworkers, and consumers; destruction of fish, birds, and other wildlife; disruption of natural biological control and pollination; extensive groundwater contamination, potentially threatening human and environmental health; the evolution of resistance to insecticides by pest species, and pest resurgence. The diversification of the approaches inherent in pest management is necessary for better environmental protection and quality storage and protection of food commodities. This includes seeking alternatives to synthetic insecticides in order to reduce insect pest damage in storage. The scientific search for botanical insecticides as cost-effective, biodegradable and eco-friendly alternatives to synthetic pesticides in smallholder agriculture has gained momentum in recent years.

Farmers in Africa have tried using plant species from over 50 families including Compositae, Fabaceae, Labiatae, Leguminoceae, Solanaceae and Umbelliferae. The most promising candidate plant materials for consideration as future grain protectants are *Azadirachta, Acorus, Chenopodium, Eucalyptus, Tephrosia, Lantana, Mentha, Ocimum, Girardia, Piper* and *Tetradenia* together with plant oils from various sources (Abete *et al*., 2007; Kamatenesi-Mugisha *et al*., 2008; Deng *et al*., 2009; Ogendo *et al*., 2011, Kariuki, *et al*., 2013). Essential oil composition is highly diverse across different plant species (Regnault-Roger *et al*., 2012). For instance, 1, 8-cineole is the major constituent of the essential oil of eucalyptus (*Eucalyptus globulus*), whereas linalool is abundant in coriander (*Coriandrum sativum*). Within the same plant species, chemo types are very common; thyme (*Thymus vulgaris*) has numerous chemo types named according to the major compound (thymol, carvacrol, terpineol, linalool) (Regnault-Roger, *et al*., 2012). Additionally, physiological expression of secondary metabolism of the plant may be different at all stages of its development. Soil acidity and climate directly affect the secondary metabolism of the plant and essential oil. This variability has important consequences on the biological activity of this essential oil and production of a standardized product, which is important for regulatory and marketing purposes (Brooker and Kleinig, 2006; Isman, 2007).

The current study has added two more species to the increasing list of insecticidal plants. The chemical constituents of *C. lusitanica* and *E. saligna* essential oils reported in the current study reveals a variation in yield and chemical composition depending on plant species and plant part sampled. Results further demonstrate that the effects of essential oils obtained from *C. lusitanica* and E. *saligna* on insect pests of stored products are manifold. They induced fumigant and contact toxicity as well as repellent effects. They were toxic to adults but also inhibited reproduction. These results are comparable to previous studies which demonstrated that plant essential oils have insecticidal, repellent and reproduction inhibition effects against various stages of stored product insect pests (Ogendo, 2008; Nerio *et al*., 2010; Alzogaray *et al*., 2011; Caballero-Gallardo, *et al*., 2012; Mishra *et al*., 2014).

The plant oils were toxic to test insects with $LC_{50}$ values of 0.05-0.11% v/w in contact toxicity, 4.07-7.02 µl/L air in fumigation and concentrations of 100 µl/L causing mortality of up to 100%, is evidence enough that the plant oils have contact and fumigant toxicity efficacy comparable to synthetic and other botanical pesticides. The recommended rate of Actelic Super $^{TM}$ (Primiphos-methyl + Permethrin) is 0.056% v/w, phosphine is 8-12 $\mu gL^{-1}$, methyl bromide is 30-50 g $M^{-3}$ grain, 50 µl $L^{-1}$ air for the highly active Labiatae species oil, ZP51 and 50-150 mg $L^{-1}$ for allyl acetate to achieve 94.0-100% mortality of all insect pests of stored cereal and legume grains (Faruki *et al*., 2005; Rajendran & Muralidharan, 2005, Ogendo *et al*., 2008b). Plant oils caused percent repellence in *T. castaneum* of 65-92.5%, and more than 30% in other test insects which is comparable to the synthetic insect repellent N, N-diethyl-*m*-toluamide (Ogendo *et al*., 2008b). The percentage progeny reduction in *C. lusitanica* and *E. saligna* oils of 50- 100% test insects was also comparable to Actelic Super $^{TM}$ (Primiphos-methyl + Permethrin).

The current study documents new milestones achieved in the investigation of the role of essential oils in stored product pest management. The study reports for the first time intra- and inter- plant variation in chemical composition of *C. lusitanica* and *E. saligna* growing in Kenya. The results of instant bioactivity (instant contact toxicity, space fumigation, instant repellence and reproductive inhibition) studies point to the achievements in studying intra- and inter-plant variations in bio-efficacy of essential oils against major coleopteran and lepidopteran of stored cereals and legumes. In addition, residual toxicity and repellence studies demonstrate the

possibly of using essential oils in the control of stored product insect pests over long storage durations under farm storage structures. Finally, suitable recommendations have been generated for further studies on more plants, insect species, and biosafety of essential oils and development of regulatory frameworks. These will in turn guide the exploitation of plant essential oils and their chemical constituents in management of stored product insect pests.

# CHAPTER 10
# CONCLUSIONS AND RECOMMENDATIONS

## 10.1 Conclusions

It may be concluded from this study that:

1. The chemical composition of *C. lusitanica* and *E. saligna* essential oils and classification into specific chemo types varied with plant species and part sampled.
2. The test essential oils exhibited weak to strong concentration- and contact time – dependent instant and residual toxicity against all the adult test insects.
3. Space and grain fumigation with *C. lusitanica* and *E. saligna* leaf essential oils resulted in moderate to strong esential oil concentration-, insect species- and fumigation duration-dependent mortality against all adult test insects.
4. The test essential oils showed weak to moderate plant species, -concentration- and contact time –dependent percent repellence (PR) against all test insects.
5. In reproductive inhibition bioassay, plant essential oils were efficient reproduction inhibitors against all test insects with significant plant-, essential oil concentration- and time- dependent progeny reduction.

## 10.2 Recommendations

As mentioned earlier (section 1.6), this study was limited by time, financial resources and laboratory equipment. Therefore, it was not practically possible to include plant part essential oils and more insect pests of stored products. Hence it is recommended that there is need to evaluate;

1. Spatial and temporal intra-plant variability in chemical composition of essential oils of *C. lusitanica* and *E. saligna*, and classification into different chemo types and bioactivity on more stored product insect pests.
2. Instant and residual contact toxicity on more plant species, essential oils, constituents combinations of the most effective essential oils and constituents and different formulations on a wider range of pests.

3. Space ad grain fumigation on more plant species , essential oils, constituents combinations of the most effective essential oils and constituents and different formulations on a wider range of pests.
4. Instant and residual repellents on more plant species , essential oils, constituents combinations of the most effective essential oils and constituents and different formulations on a wider range of pests.
5. Reproductive inhibition on more insects, plant species, essential oils, constituents combinations of the most effective essential oils and constituents and different formulations on a wider range of pests.

### 10.3 Areas for further research and policy guidelines

In order to commercialize botanical pesticides, there is also need to;

1. Evaluate socio-economic impact, biosafety, biodegradation, seed viability and quality of treated food after application of botanical insecticides.
2. Evaluate current agronomic conditions since plants are already form plantations in forests and recommend appropriate agronomic manipulations for maximize essential oil production and insecticidal efficacy.
3. Develop regulations and policies on extraction, packing and application protocols of botanical insecticides.

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# APPENDICES

Appendix 1: ANOVA output: *C. lusitanica* and *E. saligna* essential oil yield per plant and plant part.

| Source of variation | Sum of Squares | d.f | F | Prob. |
|---|---|---|---|---|
| Plants | $4.167x10^{6}$ | 1 | 0.103 | $P > 0.0\ 5$ |
| Plant part | 0.002 | 2 | 26.069 | $P < 0.001$*** |
| Plant * plant part | 0.0003 | 2 | 1.655 | $P > 0.0\ 5$ |
| Error | 0.001 | 18 | | |

Appendix 2: ANOVA output: Percent mortality of *T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais* after 24-168 h contact with five concentrations of *C. lusitanica* and *E. saligna* leaf essential oils.

| Insect/Source of variation | Sum of Squares | d.f | F ratio | Prob. |
|---|---|---|---|---|
| ***A. obtectus*** | | | | |
| Plant | 4.761 | 1 | 53.213 | $P < 0.001$*** |
| Time | 7.283 | 3 | 27.135 | $P < 0.001$*** |
| Conc. | 8.566 | 3 | 31.914 | $P < 0.001$*** |
| Plant * time | 3.509 | 3 | 13.074 | $P < 0.001$*** |
| Plant * conc. | 3.01 | 3 | 11.216 | $P < 0.001$*** |
| Time * conc. | 6.15 | 9 | 7.637 | $P < 0.001$*** |
| Plant * time * conc. | 1.871 | 9 | 2.324 | $P < 0.05$* |
| Error | 8.589 | 96 | | |
| ***S. cerealella*** | | | | |
| Plant | 0.428 | 1 | 6.071 | $P < 0.01$** |
| Time | 2.36 | 3 | 11.165 | $P < 0.001$*** |
| Conc. | 19.091 | 3 | 90.305 | $P < 0.001$*** |
| Plant * time | 0.64 | 3 | 3.029 | $P < 0.05$* |
| Plant * conc. | 1.911 | 3 | 9.039 | $P < 0.001$*** |
| Time * conc. | 4.388 | 9 | 6.919 | $P < 0.001$*** |
| Plant * time * conc. | 1.383 | 9 | 2.181 | $P < 0.05$* |
| Error | 6.765 | 96 | | |
| Total | 3327.6 | 128 | | |
| ***S. zeamais*** | | | | |
| Plant | 0.217 | 1 | 1.401 | $P > 0.05$ |
| Time | 47.494 | 3 | 102.154 | $P < 0.001$*** |
| Conc. | 16.815 | 4 | 27.126 | $P < 0.001$*** |
| Plant * time | 0.474 | 3 | 1.02 | $P > 0.05$ |
| Plant * conc. | 0.46 | 3 | 0.99 | $P > 0.05$ |
| Time * conc. | 3.579 | 9 | 2.566 | $P < 0.01$** |
| Plant * time * conc. | 1.196 | 9 | 0.858 | $P > 0.05$ |
| Error | 14.723 | 95 | | |
| ***T. castaneum*** | | | | |
| Plant | 67.577 | 1 | 293.633 | $P < 0.001$*** |
| Time | 9.115 | 3 | 13.203 | $P < 0.001$*** |
| Conc. | 59.173 | 3 | 85.706 | $P < 0.001$*** |
| Plant * time | 1.764 | 3 | 2.555 | $P < 0.05$* |
| Plant * conc. | 10.41 | 3 | 15.077 | $P < 0.001$*** |
| Time * conc. | 0.525 | 9 | 0.253 | $P > 0.05$ |
| Plant * time * conc. | 0.73 | 9 | 0.352 | $P > 0.05$ |
| Error | 22.093 | 96 | | |

Appendix 3: ANOVA output: Percent mortality of *T. castaneum*, *A. obtectus* and *S. zeamais* after 30 - 120 days contact with five concentrations of *C. lusitanica* leaf essential oils.

| Insect/Source of variation | Sum of Squares | d.f | F ratio | Prob. |
|---|---|---|---|---|
| ***A. obtectus*** | | | | |
| Exposure | 28.03 | 3 | 32.679 | $P < 0.001$*** |
| Time | 88.322 | 3 | 102.972 | $P < 0.001$*** |
| Conc. | 20.864 | 3 | 24.324 | $P < 0.001$*** |
| Exposure * time | 57.037 | 9 | 22.166 | $P < 0.001$*** |
| Exposure * conc. | 7.003 | 9 | 2.721 | $P < 0.001$*** |
| Time * conc. | 12.49 | 9 | 4.854 | $P < 0.001$*** |
| Expo * time * conc. | 8.54 | 27 | 1.106 | $P > 0.05$ |
| Error | 54.895 | 192 | | |
| ***S. zeamais*** | | | | $P < 0.001$*** |
| Exposure | 17.273 | 3 | 76.534 | |
| Time | 7.706 | 3 | 34.144 | $P < 0.001$*** |
| Conc. | 4.841 | 4 | 16.088 | $P < 0.001$*** |
| Exposure * time | 4.986 | 9 | 7.364 | $P < 0.001$*** |
| Exposure * conc. | 2.596 | 9 | 3.835 | $P < 0.001$*** |
| Time * conc. | 0.892 | 9 | 1.318 | $P > 0.05$ |
| Expo * time * conc. | 0.811 | 27 | 0.399 | $P > 0.05$ |
| Error | 14.444 | 192 | | |
| ***T. castaneum*** | | | | $P < 0.001$*** |
| Exposure | 3.449 | 3 | 9.317 | |
| Time | 8.44 | 3 | 22.8 | $P < 0.001$*** |
| Conc. | 0.975 | 3 | 2.633 | $P < 0.05$* |
| Exposure * time | 1.71 | 9 | 1.54 | $P > 0.05$ |
| Exposure * conc. | 1.595 | 9 | 1.437 | $P > 0.05$ |
| Time * conc. | 0.809 | 9 | 0.729 | $P > 0.05$ |
| Expo * time * conc. | 2.929 | 27 | 0.879 | $P > 0.05$ |
| Error | 23.69 | 192 | | |

Appendix 4: ANOVA output: Percent mortality of *T. castaneum*, *A. obtectus* and *S. zeamais* after 30 - 120 days contact with five concentrations of *E. saligna* leaf essential oils.

| **Insect/Source of variation** | **Sum of Squares** | **d.f** | **F ratio** | **Prob.** |
|---|---|---|---|---|
| ***A. obtectus*** | | | | |
| Exposure | 148.052 | 2 | 189.43 | $P < 0.001$*** |
| Time | 47.205 | 3 | 40.265 | $P < 0.001$*** |
| Conc. | 1.849 | 3 | 1.577 | $P > 0.05$ |
| Exposure * time | 20.194 | 6 | 8.613 | $P < 0.001$*** |
| Exposure * conc. | 10.916 | 6 | 4.656 | $P < 0.001$*** |
| Time * conc. | 5.11 | 9 | 1.453 | $P > 0.05$ |
| Expo * time * conc. | 4.633 | 18 | 0.659 | $P > 0.05$ |
| Error | 56.273 | 144 | | |
| ***S. zeamais*** | | | | |
| Exposure | 15.803 | 2 | 101.007 | $P < 0.001$*** |
| Time | 9.082 | 3 | 38.700 | $P < 0.001$*** |
| Conc. | 2.554 | 3 | 10.884 | $P < 0.001$*** |
| Exposure * time | 5.961 | 6 | 12.700 | $P < 0.001$*** |
| Exposure * conc. | 1.222 | 6 | 2.603 | $P < 0.01$** |
| Time * conc. | 1.335 | 9 | 1.896 | $P < 0.05$* |
| Expo * time * conc. | .941 | 18 | 0.668 | $P > 0.05$ |
| Error | 11.265 | 144 | | |
| ***T. castaneum*** | | | | $P < 0.001$*** |
| Exposure | 20.465 | 3 | 90.736 | |
| Time | 4.366 | 3 | 19.357 | $P < 0.001$*** |
| Conc. | 2.227 | 3 | 9.875 | $P < 0.001$*** |
| Exposure * time | 2.476 | 9 | 3.66 | $P < 0.001$*** |
| Exposure * conc. | 7.588 | 9 | 11.213 | $P < 0.001$*** |
| Time * conc. | 0.527 | 9 | 0.779 | $P > 0.05$ |
| Expo * time * conc. | 0.633 | 27 | 0.312 | $P > 0.05$ |
| Error | 14.435 | 144 | | |

Appendix 5: ANOVA output: Percent mortality of adult *T. castaneum*, *A. obtectus*, *S. cerealella* and *S. zeamais* after 24 h exposure to five concentrations of *C. lusitanica* and *E. saligna* leaf essential oils in space fumigation chambers.

| Insect/Source of variation | Sum of Squares | d.f | F ratio | Prob. |
|---|---|---|---|---|
| ***A. obtectus*** | | | | |
| Plant | 6.476 | 1 | 20.157 | $P < 0.001$*** |
| Time | 0.88 | 3 | 0.913 | $P > 0.05$ |
| Conc. | 328.01 | 4 | 255.227 | $P < 0.001$*** |
| Plant * time | 0.271 | 3 | 0.281 | $P > 0.05$ |
| Plant * conc. | 10.059 | 4 | 7.827 | $P < 0.001$*** |
| Time * conc. | 1.917 | 12 | 0.497 | $P > 0.05$ |
| Plant * time * conc. | 1.397 | 12 | 0.362 | $P > 0.05$ |
| Error | 38.555 | 120 | | |
| ***S. cerealella*** | | | | |
| Plant | 1.033 | 1 | 9.808 | $P < 0.01$** |
| Time | 3.608 | 3 | 11.418 | $P < 0.001$*** |
| Conc. | 28.856 | 3 | 91.313 | $P < 0.001$*** |
| Plant * time | 0.908 | 3 | 2.872 | $P < 0.05$* |
| Plant * conc. | 2.385 | 3 | 7.546 | $P < 0.001$*** |
| Time * conc. | 6.585 | 9 | 6.946 | $P < 0.001$*** |
| Plant * time * conc. | 2.198 | 9 | 2.319 | $P < 0.01$** |
| Error | 10.112 | 96 | | |
| ***S. zeamais*** | | | | |
| Plant | 0.062 | 1 | 0.126 | $P > 0.05$ |
| Time | 29.343 | 3 | 19.82 | $P < 0.001$*** |
| Conc. | 47.808 | 4 | 24.22 | $P < 0.001$*** |
| Plant * time | 0.283 | 3 | 0.191 | $P > 0.05$ |
| Plant * conc. | 11.486 | 4 | 5.819 | $P < 0.001$*** |
| Time * conc. | 12.985 | 12 | 2.193 | $P < 0.05$* |
| Plant * time * conc. | 2.367 | 12 | 0.4 | $P > 0.05$ |
| Error | 59.218 | 120 | | |
| ***T. castaneum*** | | | | |
| Plant | 7.67 | 1 | 30.607 | $P < 0.001$*** |
| Time | 8.363 | 3 | 11.124 | $P < 0.001$*** |
| Conc. | 197.502 | 4 | 197.027 | $P < 0.001$*** |
| Plant * time | 0.79 | 3 | 1.05 | $P > 0.05$ |
| Plant * conc. | 9.08 | 4 | 9.058 | $P < 0.001$*** |
| Time * conc. | 2.791 | 12 | 0.928 | $P > 0.05$ |
| Plant * time * conc. | 0.906 | 12 | 0.301 | $P > 0.05$ |
| Error | 30.072 | 120 | | |

Appendix 6: ANOVA output: Percent mortality of *T. castaneum*, *A. obtectus* and *S. zeamais* after 3-10 days grain fumigation with five concentrations of *C lusitanica* leaf essential oils.

| Insect/Source of variation | Sum of Squares | d.f | F ratio | Prob. |
|---|---|---|---|---|
| ***A. obtectus*** | | | | $P < 0.001$*** |
| Exposure | 28.009 | 3 | 106.967 | |
| Time | 5.533 | 3 | 21.131 | $P < 0.001$*** |
| Conc. | 5.261 | 3 | 20.092 | $P < 0.001$*** |
| Exposure * time | 7.221 | 9 | 9.193 | $P < 0.001$*** |
| Exposure * conc. | 7.854 | 9 | 9.999 | $P < 0.001$*** |
| Time * conc. | 2.012 | 9 | 2.562 | $P < 0.01$** |
| Expo * time * conc. | 2.511 | 27 | 1.066 | $P > 0.05$ |
| Error | 16.758 | 192 | | |
| ***S. zeamais*** | | | | $P < 0.001$*** |
| Exposure | 4.912 | 3 | 36.779 | |
| Time | 5.758 | 3 | 43.111 | $P < 0.001$*** |
| Conc. | 12.362 | 3 | 92.559 | $P < 0.001$*** |
| Exposure * time | 4.734 | 9 | 11.816 | $P < 0.001$*** |
| Exposure * conc. | 0.749 | 9 | 1.869 | $P < 0.05$* |
| Time * conc. | 0.378 | 9 | 0.944 | $P > 0.05$ |
| Expo * time * conc. | 0.706 | 27 | 0.587 | $P > 0.05$ |
| Error | 8.548 | 192 | | |
| ***T. castaneum*** | | | | $P < 0.001$*** |
| Exposure | 4.945 | 3 | 9.576 | |
| Time | 4.908 | 3 | 9.504 | $P < 0.001$*** |
| Conc. | 10.039 | 3 | 19.439 | $P < 0.001$*** |
| Exposure * time | 0.273 | 9 | 0.176 | $P > 0.05$ |
| Exposure * conc. | 9.703 | 9 | 6.263 | $P < 0.001$*** |
| Time * conc. | 0.509 | 9 | 0.329 | $P > 0.05$ |
| Expo * time * conc. | 1.018 | 27 | 0.219 | $P > 0.05$ |
| Error | 32.535 | 189 | | |

Appendix 7: ANOVA output: Percent mortality of *T. castaneum, A. obtectus* and *S. zeamais* after 3-10 days grain fumigation with five concentrations of *E. saligna* leaf essential oils.

| Insect/Source of variation | Sum of squares | d.f | F ratio | Prob. |
|---|---|---|---|---|
| ***A. obtectus*** | | | | |
| Exposure | 30.204 | 3 | 116.43 | $P < 0.001$*** |
| Time | 1.162 | 3 | 4.48 | $P < 0.01$** |
| Conc. | 77.641 | 3 | 299.294 | $P < 0.001$*** |
| Exposure * time | 2.003 | 9 | 2.573 | $P < 0.01$** |
| Exposure * conc. | 78.365 | 9 | 100.695 | $P < 0.001$*** |
| Time * conc. | 3.128 | 9 | 4.019 | $P < 0.001$*** |
| Expo * time * conc. | 5.607 | 27 | 2.402 | $P < 0.001$*** |
| Error | 16.603 | 192 | | |
| ***S. zeamais*** | | | | $P < 0.001$*** |
| Exposure | 6.201 | 3 | 23.448 | |
| Time | 8.717 | 3 | 32.963 | $P < 0.001$*** |
| Conc. | 14.981 | 3 | 56.648 | $P < 0.001$*** |
| Exposure * time | 0.939 | 9 | 1.184 | $P > 0.05$ |
| Exposure * conc. | 1.097 | 9 | 1.383 | $P > 0.05$ |
| Time * conc. | 0.536 | 9 | 0.676 | $P > 0.05$ |
| Expo * time * conc. | 1.781 | 27 | 0.748 | $P > 0.05$ |
| Error | 16.925 | 192 | | |
| ***T. castaneum*** | | | | $P < 0.001$*** |
| Exposure | 47.531 | 3 | 31.287 | |
| Time | 17.518 | 3 | 11.531 | $P < 0.001$*** |
| Conc. | 56.12 | 3 | 36.94 | $P < 0.001$*** |
| Exposure * time | 0.82 | 9 | 0.18 | $P > 0.05$ |
| Exposure * conc. | 52.22 | 9 | 11.458 | $P < 0.001$*** |
| Time * conc. | 0.661 | 9 | 0.145 | $P > 0.05$ |
| Expo * time * conc. | 9.069 | 27 | 0.663 | $P > 0.05$ |
| Error | 97.23 | 192 | | |

Appendix 8: ANOVA output: Percent repellence of adult *T. castaneum, A. obtectus, S. cerealella* and *S. zeamais* 1-24 h exposure to *C. lusitanica* and *E. saligna* leaf essential oils in untreated-treated choice bioassay system

| Insect/Source of variation | Sum of Squares | d.f | F ratio | Prob. |
|---|---|---|---|---|
| ***A. obtectus*** | | | | |
| Plant | 2.365 | 1 | 2.424 | $P > 0.05$ |
| Time | 1.261 | 3 | 0.431 | $P > 0.05$ |
| Conc. | 2.571 | 3 | 0.878 | $P > 0.05$ |
| Plant * time | 0.528 | 3 | 0.18 | $P > 0.05$ |
| Plant * conc. | 4.703 | 3 | 1.607 | $P > 0.05$ |
| Time * conc. | 4.5 | 9 | 0.512 | $P > 0.05$ |
| Plant * time * conc. | 5.288 | 9 | 0.602 | $P > 0.05$ |
| Error | 93.685 | 96 | | |
| ***S. cerealella*** | | | | |
| Plant | 22.529 | 1 | 34.586 | $P < 0.001$*** |
| Time | 2.007 | 3 | 1.027 | $P > 0.05$ |
| Conc. | 0.898 | 3 | 0.46 | $P > 0.05$ |
| Plant * time | 0.392 | 3 | 0.201 | $P > 0.05$ |
| Plant * conc. | 15.1 | 3 | 7.727 | $P < 0.001$*** |
| Time * conc. | 4.068 | 9 | 0.694 | $P > 0.05$ |
| Plant * time * conc. | 5.161 | 9 | 0.88 | $P > 0.05$ |
| Error | 62.533 | 96 | | |
| ***S. zeamais*** | | | | |
| Plant | 7.173 | 1 | 17.343 | $P < 0.001$*** |
| Time | 2.611 | 3 | 2.105 | $P > 0.05$ |
| Conc. | 7.069 | 3 | 5.698 | $P < 0.001$*** |
| Plant * time | 0.593 | 3 | 0.478 | $P > 0.05$ |
| Plant * conc. | 5.286 | 3 | 4.26 | $P < 0.01$** |
| Time * conc. | 2.778 | 9 | 0.746 | $P > 0.05$ |
| Plant * time * conc. | 4.792 | 9 | 1.287 | $P > 0.05$ |
| Error | 39.703 | 96 | | |
| ***T. castaneum*** | | | | |
| Plant | 30.128 | 1 | 63.83 | $P < 0.001$*** |
| Time | 30.445 | 3 | 21.501 | $P < 0.001$*** |
| Conc. | 17.978 | 3 | 12.696 | $P < 0.001$*** |
| Plant * time | 0.45 | 3 | 0.318 | $P > 0.05$ |
| Plant * conc. | 28.831 | 3 | 20.361 | $P < 0.001$*** |
| Time * conc. | 2.722 | 9 | 0.641 | $P > 0.05$ |
| Plant * time * conc. | 3.257 | 9 | 0.767 | $P > 0.05$ |
| Error | 45.312 | 96 | | |

Appendix 9: ANOVA output: Percent repellence of *C. lusitanica* essential oils against adult *T. castaneum, A. obtectus* and *S. zeamais* in 30 -120 days treated grain exposure duration.

| Insect/Source of variation | Sum of Squares | d.f | F ratio | Prob. |
|---|---|---|---|---|
| ***A. obtectus*** | | | | |
| Exposure | 5.897 | 3 | 3.685 | $P > 0.05$ |
| Time | 3.539 | 3 | 2.211 | $P > 0.05$ |
| Conc. | 0.987 | 3 | 0.617 | $P > 0.05$ |
| Exposure * time | 7.521 | 9 | 1.567 | $P > 0.05$ |
| Exposure * conc. | 3.661 | 9 | 0.763 | $P > 0.05$ |
| Time * conc. | 7.618 | 9 | 1.587 | $P > 0.05$ |
| Expo * time * conc. | 6.734 | 27 | 0.468 | $P > 0.05$ |
| Error | 102.418 | 192 | | |
| ***S. zeamais*** | | | | |
| Exposure | 15.681 | 3 | 10.562 | $P < 0.001$*** |
| Time | 7.138 | 3 | 4.808 | $P < 0.001$*** |
| Conc. | 19.907 | 3 | 13.409 | $P < 0.001$*** |
| Exposure * time | 1.963 | 9 | 0.441 | $P > 0.05$ |
| Exposure * conc. | 7.601 | 9 | 1.707 | $P < 0.001$*** |
| Time * conc. | 3.296 | 9 | 0.74 | $P > 0.05$ |
| Expo * time * conc. | 5.073 | 27 | 0.38 | $P > 0.05$ |
| Error | 95.015 | 192 | | |
| ***T. castaneum*** | | | | |
| Exposure | 18.046 | 3 | 9.106 | $P < 0.001$*** |
| Time | 1.56 | 3 | 0.787 | $P > 0.05$ |
| Conc. | 1.365 | 3 | 0.689 | $P > 0.05$ |
| Exposure * time | 5.398 | 9 | 0.908 | $P > 0.05$ |
| Exposure * conc. | 13.111 | 9 | 2.205 | $P < 0.05$* |
| Time * conc. | 5.785 | 9 | 0.973 | $P > 0.05$ |
| Expo * time * conc. | 15.049 | 27 | 0.844 | $P > 0.05$ |
| Error | 126.837 | 192 | | |

Appendix 10: ANOVA output: Percent repellence of *E. saligna* essential oils against adult *T. castaneum, A. obtectus* and *S. zeamais* in 30 -120 days treated grain exposure duration.

| Insect/Source of variation | Sum of Squares | d.f | F ratio | Prob. |
|---|---|---|---|---|
| ***A. obtectus*** | | | | |
| Exposure | 5.897 | 3 | 3.685 | $P < 0.05$* |
| Time | 3.539 | 3 | 2.211 | $P > 0.05$ |
| Conc. | 0.987 | 3 | 0.617 | $P > 0.05$ |
| Exposure * time | 7.521 | 9 | 1.567 | $P > 0.05$ |
| Exposure * conc. | 3.661 | 9 | 0.763 | $P > 0.05$ |
| Time * conc. | 7.618 | 9 | 1.587 | $P > 0.05$ |
| Expo * time * conc. | 6.734 | 27 | 0.468 | $P > 0.05$ |
| Error | 102.418 | 192 | | |
| ***S. zeamais*** | | | | |
| Exposure | 15.681 | 3 | 10.562 | $P < 0.001$*** |
| Time | 7.138 | 3 | 4.808 | 0.003 |
| Conc. | 19.907 | 3 | 13.409 | $P < 0.001$*** |
| Exposure * time | 1.963 | 9 | 0.441 | $P > 0.05$ |
| Exposure * conc. | 7.601 | 9 | 1.707 | $P < 0.05$* |
| Time * conc. | 3.296 | 9 | 0.74 | $P > 0.05$ |
| Expo * time * conc. | 5.073 | 27 | 0.38 | $P > 0.05$ |
| Error | 95.015 | 192 | | |
| ***T. castaneum*** | | | | |
| Exposure | 18.046 | 3 | 9.106 | $P < 0.001$*** |
| Time | 1.56 | 3 | 0.787 | $P > 0.05$ |
| Conc. | 1.365 | 3 | 0.689 | $P > 0.05$ |
| Exposure * time | 5.398 | 9 | 0.908 | $P > 0.05$ |
| Exposure * conc. | 13.111 | 9 | 2.205 | $P < 0.05$* |
| Time * conc. | 5.785 | 9 | 0.973 | $P > 0.05$ |
| Expo * time * conc. | 15.049 | 27 | 0.844 | $P > 0.05$ |
| Error | 126.837 | 192 | | $P > 0.05$ |

Appendix 11: ANOVA output: Percent reduction of progeny of *T. castaneum, A. obtectus* and *S. zeamais* within 5-20 days emergence time (days) after grains were treated with concentrations of 0.05 -0.20 % v/w of *C. lusitanica* and *E. saligna* essential oils.

| **Insect/Source of variation** | **Sum of Squares** | **d.f** | **F ratio** | **Prob.** |
|---|---|---|---|---|
| ***A. obtectus*** | | | | |
| Plant | 0.125 | 1 | 31.621 | $P < 0.001$*** |
| Time | 0.049 | 3 | 4.164 | $P < 0.01$** |
| Conc. | 0.234 | 3 | 19.756 | $P < 0.001$*** |
| Plant * time | 0.079 | 3 | 6.639 | $P < 0.001$*** |
| Plant * conc. | 0.128 | 3 | 10.77 | $P < 0.001$*** |
| Time * conc. | 0.084 | 9 | 2.358 | $P < 0.05$* |
| Plant * time * conc. | 0.153 | 9 | 4.311 | $P < 0.001$*** |
| Error | 0.38 | 96 | | |
| ***S. zeamais*** Plant | 0.188 | 1 | 3.197 | $P < 0.05$* |
| Time | 4.143 | 3 | 23.54 | $P < 0.001$*** |
| Conc. | 6.064 | 3 | 34.451 | $P < 0.001$*** |
| Plant * time | 0.2 | 3 | 1.134 | $P > 0.05$ |
| Plant * conc. | 0.365 | 3 | 2.075 | $P > 0.05$ |
| Time * conc. | 0.21 | 9 | 0.398 | $P > 0.05$ |
| Plant * time * conc. | 0.061 | 9 | 0.116 | $P > 0.05$ |
| Error | 5.633 | 96 | | |
| ***T. castaneum*** Plant | 0.439 | 1 | 1.843 | $P > 0.05$ |
| Time | 12.976 | 3 | 18.139 | $P < 0.001$*** |
| Conc. | 1.228 | 3 | 1.717 | $P > 0.05$ |
| Plant * time | 1.135 | 3 | 1.587 | $P > 0.05$ |
| Plant * conc. | 1.33 | 3 | 1.859 | $P > 0.05$ |
| Time * conc. | 2.263 | 9 | 1.055 | $P > 0.05$ |
| Plant * time * conc. | 1.209 | 9 | 0.564 | $P > 0.05$ |
| Error | 22.893 | 96 | | |

Appendix 12: Publications

(a) Peer-reviewed papers and conference proceedings

1. **Bett, P.K.,** Deng, A.L., Ogendo, J.O., Torto, B., Kamatenesi-Mughisha, M., Mihale, J.M. Kariuki, S.T. 2014. *Essential oils of Cupressus lusitanica and Eucalyptus saligna as fumigants of stored product insect pests: The hope of the future for small-scale farmers*. Extended Abstracts 4th RUFORUM (Regional Universities Forum for Capacity Building in Agriculture) Biennial Conference, 21-25 July 2014, Maputo, Mozambique.
2. **Bett, P.K.,** Deng, A.L., Ogendo, J.O., Kamatenesi-Mughisha, M., Mihale, J.M. 2013.Toxic and repellent properties of *Cupressus lusitanica* and *Eucalyptus saligna* essential oils against *Callosobruchus chinensis* and *Sitophilus zeamais.* In: *Proceedings of the First International Conference on Pesticidal Plants* Volume 1 (August, 2013), Ogendo, J. O., Lukhoba, C. W., Bett, P. K., Machocho, A. K. (ed.). pp 121-123, ADAPPT-Network: Egerton University, Kenya.
3. Ogendo, J.O., Deng A. L., Birech, R. J. and **Bett, P. K.** 2012. Plant-Based Products as Control Agents of Stored-Product Insect Pests in the Tropics, in: *Progress in Food Preservation***,** Bhat, R., Alias, A.K. and Paliyath, P. (Ed.). Wiley-Blackwell: London, pp. 581-601.
4. **Bett, P. K**., Deng, A. L., Ogendo, J. O., Torto, B., Mugisha-Kamatenesi, M. Mihale, J.M. 2011. Chemical composition and insecticidal activity of the leaf essential oil of *Eucalyptus saligna* against *Acanthoscelides obtectus* say (Bruchidae) and *Sitotroga cerealella* Olivier (Gelechiidae). *Book of abstracts*, sixth Egerton University International Conference: Research and Expo. 21$^{st}$ – 23$^{rd}$ September 2011 Agriculture Resources Centre, Egerton University, Njoro, Kenya. pp.38-39
5. Deng, A.L., Ogendo, J.O., Owuor, G., **Bett, P.K.,** Omolo, E.O., Mugisha-Kamatenesi M. Mihale, J.M. 2009. Factors determining the use of botanical insect pest control methods by small-holder farmers in the Lake Victoria Basin, Kenya. *African J. of Environmental Science and Technology* **3**(5): 108-115.

(b) Submitted manuscripts under review

1. Chemical composition of *Cupressus lusitanica* and *Eucalyptus saligna* leaf essential oils and bioactivity against major insect pests of stored food grains. *Industrial Crops and Products.*
2. Hydro-distillation and GC/-MS analysis of volatile constituents of Kenyan *Cupressus lusitanica* Miller. *Phytochemical Analysis*.